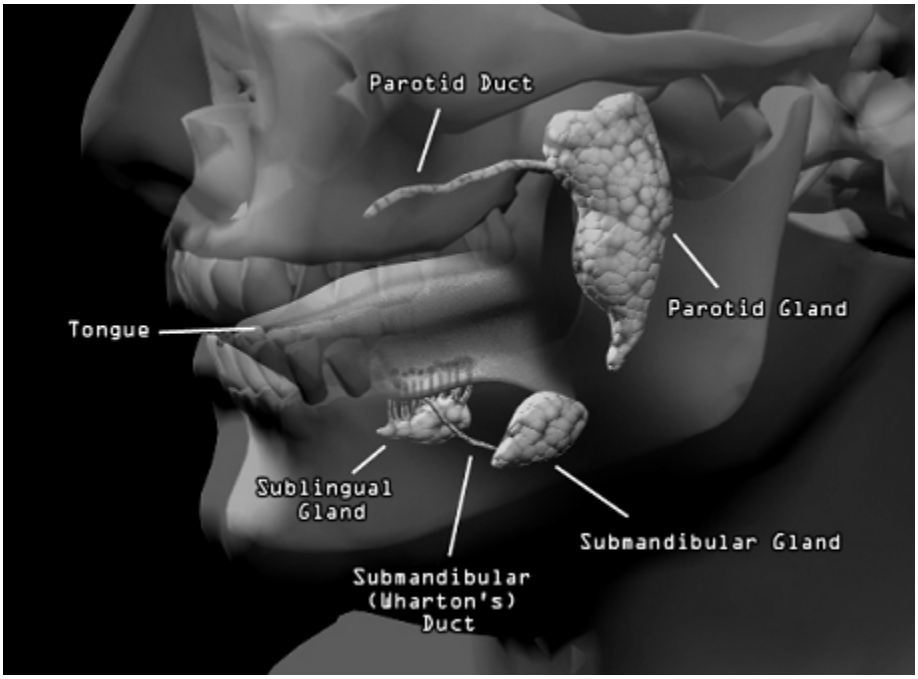
The Importance of Mouth Location During Saliva Collection

SPITTIPS

To most people all saliva appears to be pretty much the same. To oral biologists, however, saliva is a fascinating mixture of several fluids made by different types of salivary glands in different areas of the mouth. Saliva from all of the glands contains certain common components, but concentrations of certain other components can vary significantly from one type of gland to another. For this reason, when saliva is to be tested for these analytes that can be affected, it is important that the differences are understood and that the proper collection location in the mouth is consistently used.

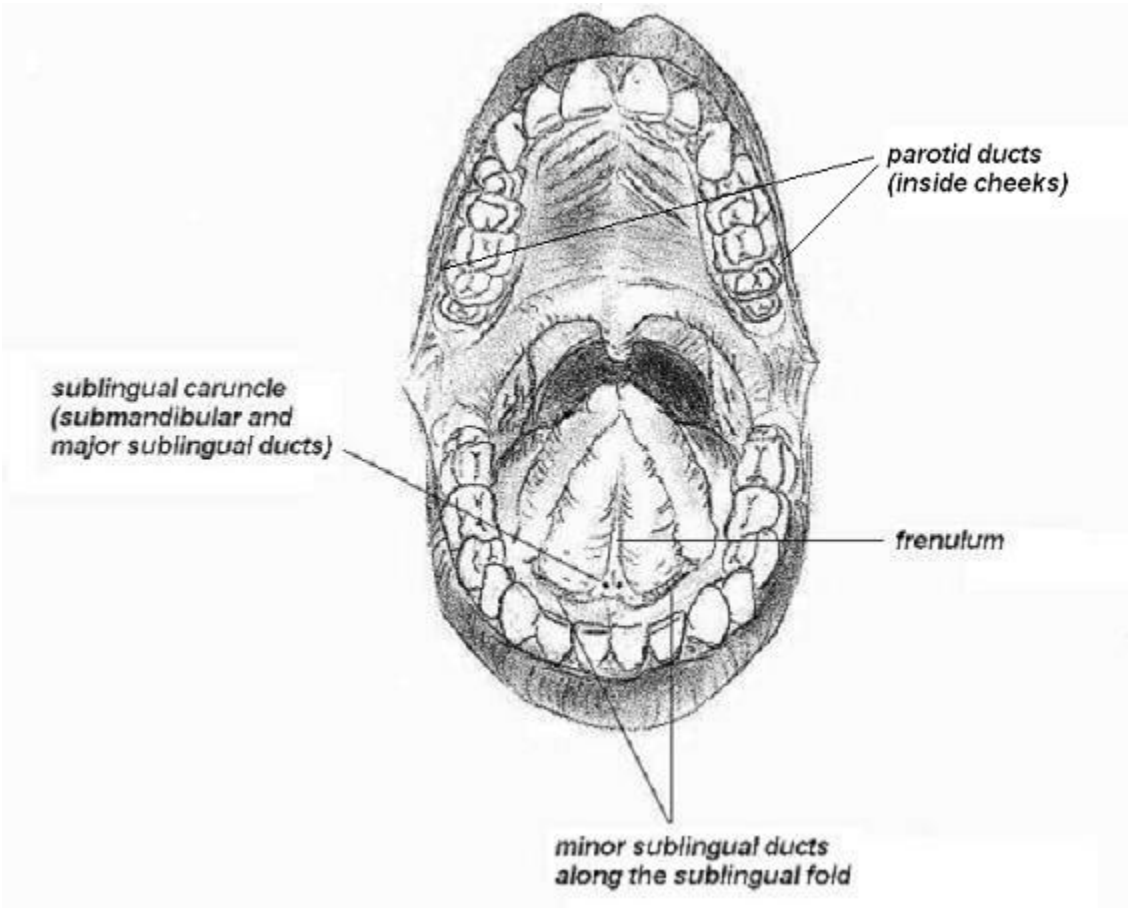
Locations of the Salivary Glands and Ducts

Most of the saliva in the mouth is secreted by three pairs of major glands: the parotid, the submandibular, and the sublingual. These are located symmetrically on either side of the mouth.



- The parotid glands empty through the *parotid ducts,* which open into the cheeks adjacent to the second upper molars.
- Each submandibular gland empties into one long duct, the *submandibular (or Wharton's) duct*, which opens at the *sublingual caruncle* underneath the tongue. The openings from the two ducts are found just to either side of the frenulum.

- The sublingual glands are different in that they do not empty into a single duct. Rather, the front portion of each gland empties into the *major sublingual duct.* This duct sometimes opens adjacent to the *submandibular duct*, or in some individuals it merges with the *submandibular duct* just before reaching the mouth. The rear portion of each sublingual gland empties through 10-12 *lesser sublingual ducts.* These short ducts open directly upwards in a row through the floor of the mouth along the sublingual fold, which runs obliquely from the sublingual caruncle off to either side.



In addition to the major salivary glands, there are hundreds of minor glands located in the lips, tongue, palate, and cheeks. Unlike the major glands, the minor glands do not form large structures with branched ducts, and each gland empties directly through its own small duct.

The Importance of Mouth Location During Saliva Collection

Production of Saliva

The secretory units of the saliva glands are made up largely of two types of secretory cells, serous and mucous. These cells form globular or tubular clusters known as the acini. The acinar cells import water, salts, and various other components derived from plasma and combine them to produce saliva. The acinar cells also use some of the raw materials for the synthesis of large quantities of proteins that are added to the saliva. (1)

Saliva glands also contain duct cells. In addition to delivering the saliva to the mouth, duct cells also move ions in and out of the salivary product to finalize its composition. Certain types of duct cells can also synthesize additional proteins that are added to the saliva.

For further information about the saliva glands, salivary cells, and the mechanism of saliva production, please see the article *Saliva and Its Use as a Diagnostic Fluid* on our website.

Characteristics of Glandular Salivas

Each type of salivary gland is composed of a different ratio of serous and mucous cells, and there are also differences in the types of duct cells present. Since some salivary components are produced by only one type of cell, the composition of saliva produced by each gland is affected by the cellular composition of the gland. (2)

- The parotid glands are made up almost exclusively of serous acini. They produce a watery type of saliva containing large amounts of the digestive enzyme α-amylase and proline-rich proteins, plus lower levels of other components such as cystatins and histatins.
- The sublingual glands are largely made up of mucous cells, with small numbers of serous cells. The saliva produced is viscous due to high concentrations of glycoproteins known as mucins, and it also contains high levels of the enzyme lysozyme.
- The submandibular glands contain both types of cells, with the serous somewhat more numerous. Submandibular saliva is therefore a mixture of the thick and thin types. It contains α-amylase, but at a lower level than in parotid saliva, along with mucins and other components derived from the mucous cells. It also contains the highest concentration of salivary cystatin C.
- Most of the minor salivary glands are mucous in nature, and most often they produce saliva that is similar to that of the sublingual glands. There is some variation found, however. For example, the von Ebner's glands located on the back of the tongue secrete a serous product rich in the digestive enzyme lipase

Whole Saliva

Whole saliva is a mixture of all the various glandular salivas produced in the mouth, along with varying amounts of other components such as tears, nasal and bronchial secretions, bacteria (and products derived from them), blood products from microinjuries or oral disease, and the serum-like gingival crevicular fluid (GCF) from the junction between teeth and gums. The composition of whole saliva is not constant, and it changes depending on the degree of stimulation present in the various glands.

For example, in the rest state, with minimal stimulation, the submandibular glands are the most active, producing around 65% of the saliva in the mouth. Whole saliva in this state is rich in mucins derived from the mucous cells of the sublingual, submandibular, and minor glands. Once stimulation from taste, smell, or chewing motions of the jaw activates the parotid glands, however, they quickly increase saliva production, creating large amounts of watery saliva containing enzymes like α-amylase and lipase. (3) This increased flow helps chewing and also begins the digestive process. As a result of increased parotid flow, the concentration of the mucins is reduced in the whole saliva, and the balance of other components may also be altered.

Controlling for Variability in Saliva Testing

Given the differences that can exist in the composition of saliva from one gland to another, and even from an individual gland over time, it is important that investigators take steps to collect saliva samples consistently in a manner that is appropriate for the analytes in their studies. To illustrate, let us consider three biomarkers commonly used in stress research: cortisol, α-amylase, and SIgA.

Cortisol

Cortisol is the principal steroid hormone produced in the adrenal cortex in response to stress through the HPA axis. It circulates in the blood system, and it enters saliva quickly and easily by passive diffusion through the cells of the salivary glands. Studies have shown that levels of salivary cortisol are highly correlated to serum levels, and it is widely accepted as an excellent measure of the unbound, bio-available cortisol in the bloodstream. (4) Cortisol appears to enter equally well into the different types of salivary glands, and it is an example of a biomarker that is not significantly affected by the location where the saliva is collected.

α-Amylase

α-Amylase is an example of a protein manufactured locally in the acini of the salivary glands. It is best known as a digestive enzyme that degrades starch molecules, but it also binds to tooth surfaces and bacteria and plays a role in the maintenance of oral biofilms. After synthesis, α-amylase is stored in the secretory cells, then released into saliva by nervous signals. Recent research has demonstrated that its concentrations in saliva can be used as a convenient measure of activity in the autonomic nervous system. (5)

As noted above, α-amylase concentrations are higher in pure parotid saliva than in whole saliva or in the other glandular salivas. For this reason, it is important that a consistent collection location is used throughout each study. For studies where α-amylase will be measured along with other analytes, we recommend collecting unstimulated whole saliva by the passive drool technique described below. Alternatively, studies that focus on α-amylase alone may want to collect samples of parotid saliva, which can easily be done with the Salimetrics Oral Swab (SOS), as described below.

SIgA

Secretory IgA is an important immunoglobulin, often referred to as the first line of defense against invading pathogens. IgA is synthesized by plasma cells scattered among the salivary acini and ducts, then actively transported through the cell membranes by a Polymeric Immunoglobulin Receptor (PIgR) and released into the saliva as Secretory IgA (SIgA).

The levels of SIga found in saliva appear to be tied to the relative numbers of IgA producing plasma cells associated with each type of salivary gland. The submandibular glands have approximately twice the number of plasma cells per unit tissue weight than do the parotid glands, and they have a correspondingly larger output of SIgA. (6) The minor salivary glands have even higher numbers of plasma cells, and their saliva has been reported to contain four times as much SIgA as parotid saliva. (7) SIgA is therefore a good example of an analyte where consistency in the choice of collection location is extremely important.

Collection Methods

For many studies, and especially for those that will archive samples for future possible use to test multiple analytes, we recommend collecting unstimulated whole saliva that pools on the floor of the mouth, using the passive drool technique.

Alternatively, some people find it easier and aesthetically more acceptable to collect saliva by placing an absorbent device in the mouth. We recommend our own Salimetrics Oral Swab (SOS), which is made of an inert polymer shaped into a 30 x 10 mm cylindrical roll. (Note that some analytes are affected by the use of absorbent devices, however.) For infants and small children we have developed the Salimetrics Infant's Swab (SIS) and the Salimetrics Children's Swab (SCS), which are made of the same polymer as the SOS. These devices have smaller diameters that are better suited to the mouths of children and longer lengths, which allow an adult to hold one end while the other end is placed in the mouth of the child, in order to avoid the hazard of choking. Detailed protocols for all of these collection devices are available in our *Saliva Collection and Handling* booklet, which may be downloaded from our website.

When saliva is collected by placing the swab underneath the tongue on the floor of the mouth, we find that assay results are similar to those from whole saliva collected by passive drool. Under certain conditions, however, there is a possibility that the swab might collect specific glandular saliva, rather than whole saliva. For this reason we recommend that these absorbent devices be used in a consistent manner under the front of the tongue and that they not be moved around in the mouth, especially if any of the analytes being measured is known to be affected by mouth location.

Alternatively, for more specialized studies, the SOS can also be used to collect samples of parotid saliva by placing the device between the cheek and upper gum next to the second upper molar, where the parotid duct opens into the mouth. Due to the lower flow rate of unstimulated parotid saliva, however, the device should be left in place for a longer period to ensure that adequate sample is collected.

Conclusion

Before beginning to collect saliva for a study, we strongly advise studying the literature and consulting with us in order to understand the effects that mouth location and collection technique may have on the analytes to be measured. Once the desired protocol has been established, it is then important to take steps to educate donors so that they understand and follow the proper collection technique. For those analytes that are easily affected by the type of saliva collected, it may be advisable not to allow unsupervised self collection in the home. By following these steps, researchers will be rewarded with higher quality data that may make it easier to observe relationships that might otherwise have been obscured.

References

1. Boron, W.F., & Boulpaep, E.L. (2003*). Medical physiology: A cellular and molecular approach.* Philadelphia, PA: Saunders (Elsevier Science).
2. Veerman, E.C., van den Keybus, P.A., Vissink, A., & Nieuw Amerongen, A.V. (1996). Human glandular salivas: Their separate collection and analysis. *Eur J Oral Sci*, *104*(4), 346-52.
3. Humphrey, S.P., & Williamson, R.T. (2001). A review of saliva: Normal composition, flow, and function. *J. Prosthet Dent, 85*(2), 162-69.
4. Vining, R.F., McGinley, R.A., Maksvytis, J.J., & Ho, K.Y. (1983). Salivary cortisol: A better measure of adrenal cortical function than serum cortisol. *Ann Clin Biochem, 20*(6), 329-35.
5. Nater, U.M., Rohleder, N. (2009). Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: Current state of research. *Psychoneuroendocrinology*, *34*(4), 486-96.
6. Brandtzaeg, P. (2007) Do salivary antibodies reliably reflect both mucosal and systemic immunity? *Ann N Y Acad Sci, 1098,* 288-311.
7. Crawford, J.M., Taubman, M.A., & Smith, D.J. (1975). Minor salivary glands as a major source of secretory immunoglogulin A in the human oral cavity. *Science, 190*(4220), 1206-9.