

# Oral Fluid in Toxicology

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## Abstract

Saliva is a combination of gingival crevicular fluids, fluid of salivary glands. The most commonly used laboratory diagnostic procedures involve the analysis of cellular and chemical constituents of blood. Other biologic fluids are utilized for diagnosis of disease, and saliva offers some distinctive advantages. Whole saliva can be collected non-invasively and by individuals with limited training. This article reviews role of saliva in toxicology.

## INTRODUCTION

Whole saliva is a combination of gingival crevicular fluid, which has a composition similar to serum, and fluid released from salivary glands, of which the parotid, submandibular and sublingual are the three major sources.<sup>1</sup> The components of saliva are water, proteins, electrolytes, organic molecules secreted from salivary glands, blood, microbes, epithelial lining cells, extrinsic factors and some additional fluids. The most commonly used laboratory diagnostic procedures involve the analysis of the cellular and chemical constituents of blood. Other biologic fluids are utilized for diagnosis of disease, and saliva offers some distinctive advantages. Whole saliva can be collected non-invasively, and by individuals with limited training. In recent years saliva has attracted much attention, in particular among people interested in the determination of drug concentrations. This suggests that saliva might be substituted for plasma in the areas of pharmacokinetic studies and drug monitoring because of the growing interest in non-invasive procedure.

## PHYSIOLOGY AND BIOCHEMICAL ASPECTS

**Salivary secretions:** Saliva is a complex oral fluid consisting of a mixture of secretions from both major salivary glands as well as minor glands of oral mucosa. Once saliva passes through ducts and enters the oral cavity, it mixes with blood cells, micro-organisms and microbial products; oral epithelial cells and cell products, food debris and upper airway secretions.

The human salivary glands produces about 600 ml/day of serous and mucinous saliva containing minerals, electrolytes, buffers, enzymes and enzyme inhibitors, growth factors and cytokines, immunoglobulins, mucins and other glycoproteins.<sup>2,3,4,5</sup> Proteins that are found in saliva such as

lactoferrin, lysozyme peroxidase, defensins and histatins, can destroy or inhibit the growth of micro-organisms in oral cavity.<sup>6</sup>

**Composition of saliva:**

pH: 6.4-7.1 alkaline, viscous secretion water content 99.5% solid content 0.5% (40% inorganic constituents / 60% organic constituents): Organic constituents: -> Mucin: -> Enzymes: -> -> Amylase: -> -> Lysozyme: -> -> Albumin, globulin: Others urea, uric acid, cholesterol, vitamins, phospholipids

**Mucin:** Mucin is a glycoprotein, insoluble in water of dilute acid and gives viscosity to saliva.

**Microscopy of saliva reveals**

Epithelial cells  
Salivary corpuscles  
Mucus food debris

**Microbes:** Bacteria, fungi and protozoa.

Salivary composition depends on many factors: stimulation, diet, age, time of day, disease etc. Ordinary saliva varies weakly alkaline to weakly acid, the pH ranging approximately 6.0-7.9 with optimum pH of 6.6. Lower pH values occur more frequently among caries susceptible individuals and dental erosion is often accompanied by greatly increased total salivary acidity. Saliva is a dilute secretion with specific gravity of 1.007. Normal saliva contains glucose, potassium thiocyanate and cyanate which possibly comes from ingested cyanides present in certain fruits, in tobacco smoke and from breaking down of protein material. Apoerytheine, a protein fraction that protects

vitamin B12 from digestive destruction is also present in saliva. The amount of saliva secreted by an adult in 24 hours varies between 1000 and 1500 ml.<sup>3</sup> In the absence of obvious external stimuli, the rate of salivary secretion in adult is between 0.1 ml & 0.25 ml per minute and values < 0.1 l/min should be considered abnormal. The stimulated flow rate varies between 1-2 ml/min and values < 0.5 ml/min should be considered abnormal.<sup>3</sup> Saliva is routinely categorized as resting (unstimulate) or stimulated. The resting saliva reflects the basal flow rate and it is present in our mouths coating the oral tissues about 14 hours of the day. Stimulated saliva is also protective and is present in our mouths for upto about 2 hours of the day.

### REGULATION OF SALIVARY GLAND SECRETIONS

Secretion of saliva is governed by central nervous system along with sympathetic nervous system. No hormone mechanism in salivary secretion is known. Ordinarily, the secretion of saliva is the result of reflex stimulation of secretory nerves through a center in medulla oblongata, psychic stimuli brought about by such as thought of food, also stimulate its secretion the control of salivary secretion is exclusively neural. The flow rate of saliva during sleep is small. This spontaneous secretion keeps mucous membrane moist. Stimulated secretion occurs via nervous reflexes. Neural mechanoreceptors and chemoreceptors in the oral cavity respond to dryness of mucosa, chewing chemical in foods and texture of the food. Afferent impulses are integrated in medulla and salivary center receives inputs from cortex, amygdala and hypothalamus.

Salivary gland secretions may be inhibited temporarily with infections or drugs. Permanent inhibition occurs in irradiation of head and neck, Sjogren's syndrome and is primarily associated with alimentary functions of saliva.

### SALIVA: SAMPLE COLLECTION, PRESERVATION AND PRETREATMENT FOR ANALYTICAL TECHNIQUES,,,

Saliva is ultrafiltrate of plasma. In a clinic or lab, saliva is relatively easy to collect in sufficient quantities for analysis and the costs of storage and shipping tend to be lower than those for serum and urine. Saliva is easy to obtain, with less invasion of privacy and ease of adulteration, compared to urine. Salivary sampling protocols are advantageous as they make for frequent and easy collection of samples by non-invasive NEEDLE-FREE stress free techniques.

### ADVANTAGES:

Saliva measures free, bioavailable fraction of steroid hormones and drug that have moved out of bloodstream and into the tissue.

- Most reliable measurement of tissue uptake in case of topical hormone supplement.
- Painless, non-invasive, needle free.
- Private, convenient for both patient and doctor.
- Transportation of saliva samples to laboratory requires no special handling.
- Less expensive than conventional blood testing.
- Ease of collection allows for routine monitoring and adjustment of hormone supplement if required.

### DRAWBACKS OF USE OF SALIVA

Most of the drugs are present in blood and usually the concentration of drugs tested are higher in blood than in saliva.

Forensic toxicologists know little about saliva and are understandably reluctant to use it as a drug determinant fluid.

Forensic laboratories are now automated with setting or their mechanisms food blood and urine. Setting them up for saliva will be required.

### SAFETY

For forensic scientists, saliva tests are safer than blood tests, which are more likely to result in exposure to HIV or hepatitis. For the patient, from invasive collection techniques to saliva can dramatically reduce anxiety and discomfort, thereby simplifying collection of serial samples for monitoring of drug.

### COLLECTION

Different techniques have been devised for the collection of saliva. Usually, an individual is asked to rinse out his mouth with water and then chew an inert material such as a piece of rubber or paraffin wax from 30 seconds to several minutes. The first mouthful of saliva is discarded; there after the saliva is collected into a small glass bottle.

- Also saliva can be absorbed onto a swab.
- Non-invasive home testing of saliva; saliva kits are

available or collection of specific gland saliva.

- In case of edentulous, 2% citric acid is employed to obtain stimulated saliva.
- Collection of saliva may be difficult from individuals who experience: anticholinergic symptomatology and alcoholics.

### SALIVA COLLECTION DEVICE AVAILABLE

Salivette, omnisal, orasure

Saliva is allowed to pool in the bottom of the mouth and collected into a plastic vial centrifuged at 3000 rpm for 5-10 minutes and supernatant fraction is stored at  $-20^{\circ}\text{C}$  or  $-80^{\circ}\text{C}$  until analyzed.

### SPLIT KIT

Split kits are available and contain:

- plastic disposable to collect saliva
- standardized piece of paraffin wax or unflavoured sugarless chewing gum
- pH paper
- strip to assess buffer capacity of saliva
- material for microbiological test

### PRESERVATION

Once the samples have been collected, it is important that they be properly stored unless analyses are to be performed immediately. For long term storage of salivary samples at room temperature required cortisol.<sup>16</sup> Quickly freeze them on collection with thawing and centrifugation, glycoproteins in saliva precipitate out, leaving behind a pipettable clear fluid. In forensic work in which saliva samples have been taken primarily for serological purposes, it is common practice to subject the sample and container to boiling water temperatures for 15-30 minutes prior to freezing. Only in cases in which the toxic material present in saliva is volatile or heat unstable would this treatment be expected to be deleterious to later analysis of such saliva samples. Another point where opinions differ about is the difference in point of time in which the pH of the saliva samples should be measured, either immediately after collecting the sample or before analysis of the sample. Probably when the researchers measure pH immediately after collection they use the pH to clarify the transport mechanism. However, when pH is

measured after using the pH is used for analytical procedure.

### OTHER DRUGS

Other drugs that can be identified in saliva such as amphetamines, barbiturates, benzodiazepines, phenylclidine, cocaine, opioids.<sup>32,33</sup> Saliva can be used to detect recent marijuana use by means of radio immunoassay<sup>34</sup>. A major psychoactive component of marijuana, can be detected in saliva for at least 4 hours after marijuana is smoked.<sup>35</sup> Saliva can be used to monitor tobacco smoking and exposure to tobacco smoke. Salivary cotinine levels were found to be indicative of active and passive smoking.<sup>36,37</sup>

### DOPING DRUGS

The most frequently used biological specimen for the determination of drugs in doping control is urine, since only a non-invasively obtained sample is acceptable for routine collection. Yet, even the acceptability of urine sample is being disputed in view of the potential invasion of privacy, especially if a directly observed collection is advisable to prevent adulteration or substitution of sample.<sup>38</sup> That happens, for instance, when athletes try to escape detection by using urine from someone else. Another major disadvantage of urine is the variability in the renal clearance of drugs and their metabolites, which is largely due to fluctuations in the flow rate and pH of urine. Not all drugs are excreted in the urine, for instance, the lipidsoluble  $\beta$ -blocking drugs tend to be rapidly eliminated by various metabolism systems in the liver.<sup>39</sup> At present, saliva is not used as a biological fluid for doping control. Although a qualitative doping control mainly depends on the sensitivity of the assay, the usefulness of saliva needs to be explored here further.

### CONCLUSION

In drug analysis, research involving the use of saliva sampling as non-invasive qualitative and quantitative techniques has become increasingly important. Saliva offers distinctive advantages over serum because it can be collected non-invasively by individuals with modest training, because of the growing interest in non-invasive procedures; this updated review evaluates the use of saliva in drug analysis and in therapeutic and toxicologic monitoring. So far, no device could be found to serve for all saliva analyses. Although highly sensitive methods of detection are required, most drugs can be detected in salivary secretions. Further studies will be required for proper relation between saliva and each drug. Also, saliva can be used as a tool in toxicology.

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