

Methods of collection of saliva - A Review

Yamuna Priya K¹, Muthu Prathibha K^{2,*}

¹Consultant, Sai Dental Clinic, Thiruvapur, ²Associate Professor, Dept. of Physiology, Saveetha Medical College, Thandalam, Chennai

***Corresponding Author:**

Email: drmuthuprathi@gmail.com

Abstract

In the recent days, saliva has gained importance as a diagnostic fluid. Human saliva is an easily accessible biochemical fluid, similar to blood in various biological aspects. When compared to other body fluids, saliva is readily available, can be non-invasively collected, with no risk of cross infection. Moreover, salivary analysis can detect changes that are reflective of both oral and systemic conditions. Despite its advantages as a diagnostic fluid, lack of adequate and accurate information regarding the methods of collection and storage of samples, prior to the analysis hampers the frequent use of saliva. Successful measurement of salivary parameters requires optimal collection procedures, which is one of the most important pre analytical steps. The present paper is a brief review of the most common and recent methods for collection of saliva. The authors have focussed on the techniques, requisites, advantages, disadvantages and possible variations that can affect the collection of saliva.

Introduction

Oral samples that are useful for the diagnosis of systemic and oral diseases include saliva, gingival crevicular fluid (GCF), oral swabs, dental plaque and volatiles. Recent years has seen an increasing interest in the use of oral fluids as diagnostic tools, especially whole or total saliva. The incorporation of salivary diagnostics into clinical practice is gaining reality and will be of diagnostic value in the prospective future. The major (sub- mandibular, parotid and sublingual) and minor salivary glands together produce about 90% and 7% of saliva respectively.⁽¹⁾ Daily secretion of saliva ranges between 500 – 700ml.⁽²⁾ At rest, secretion ranges from 0.25 to 0.35 ml/min and is mostly produced by the submandibular and sublingual glands. Stimulation can raise the secretion rate to 1.5 ml/min.⁽²⁾ The greatest volume of saliva is produced before, during and after meals, reaching its maximum peak at around 12 am., and falls considerably at night, during sleep.⁽²⁾ Saliva has many important roles in oral health like lubrication, physical protection, cleansing, buffering, tooth integrity, maintenance of pH and antibacterial action.

Several authors have reported significant correlations between the composition of certain substances in saliva and blood. Saliva appears to be a promising diagnostic fluid in the monitoring of general public health, early disease detection, bio monitoring of pesticides, monitoring of drugs and smoking and various forensic and clinical applications.⁽³⁾ Also, saliva has gained importance as a diagnostic tool because it is readily available and can be non- invasively collected with no major risk of cross infections. It detects oral and systemic illnesses at an early stage and is quite representative of various changes occurring in the body.⁽⁴⁾ Saliva in normal quantity and composition, is likely to be the most important aspect of the host immune system in the protection of oral cavity from disease.⁽⁵⁾ However, systemic diseases like Sjogren syndrome,

Systemic Lupus erythematosus, systemic sclerosis, rheumatoid arthritis, diabetes, cystic fibrosis, HIV etc. are associated with salivary gland dysfunction.^(5,6)

Physical parameters like salivary pH, buffering capacity, flow rate, viscosity and chemical assays like salivary hormones, antibodies and tumour markers are gaining importance in the diagnosis of many systemic disorders. The lack of understanding of the diagnostic uses of saliva and non availability of adequate details of the methods of collection of saliva have hampered the use of saliva earlier. There have been repeated attempts by several authors to address these challenges. Numerous methods have been evaluated by several authors for the collection of saliva. The accurate measurements of salivary flow rate and its composition requires optimal collection, processing and storage. The materials and techniques used to collect, store and analyse the salivary samples exert a significant influence on the accuracy of testing. Hence, the authors planned to review the existing methods available for collection of saliva with an additional focus on the advantages, disadvantages and precautions to be taken in this regard.

Saliva can be collected in different forms - a) resting or unstimulated whole saliva, b) stimulated whole saliva, c) glandular saliva (mainly parotid) - with or without stimulation, sub-mandibular/ sub-lingual saliva, d)palatine saliva. Whole saliva is composed of secretions from salivary gland as well as from GCF, desquamated epithelial cells, microorganisms and leukocytes. Normal whole saliva secretion varies between 800- 1500 ml / day or 1.0 to 3.0 ml / minute with a pH in the range of 6-7 for unstimulated whole saliva.^(5,8)

Unstimulated saliva is the mixture of secretions which enters the mouth in the absence of exogenous stimuli. It reflects the basal salivary flow rate, present in the oral cavity for about 24 hours a day. In salivary diagnostics, unstimulated saliva is often preferred to the stimulated whole saliva, since the latter contains only a

diluted concentration of biomarkers, that may be difficult to detect.⁽⁷⁾ Nevertheless, the degree of hydration, body posture and position of head during collection, exposure to light, drugs and circadian rhythm affect the unstimulated saliva.⁽⁸⁾

Stimulated saliva is secreted in response to either masticatory or gustatory stimulations. Factors affecting stimulated saliva include gland size, food intake, smoking, gag reflex and type of stimulation given. Stimulated saliva represents the secretion during food intake (physiological stimulation), and is present in the mouth for up to 2 hours.⁽³⁾ Various stimulants like paraffin wax, unflavoured chewing gum base, cotton puff and rubber bands bring about masticatory stimulation, whereas citric acid and sour candy drops result in gustatory stimulation. Gustatory stimuli have been found to exert a greater effect on salivary composition than masticatory stimulants. Mechanical (masticatory) stimulation will not interfere with saliva composition; however, it is difficult to maintain a constant force of stimulation (mastication) through-out the collection period. Standard-size gum base or paraffin wax (1.5 g, mp = 42 °C) can be used as a stimulant. The frequency of stimulation can be controlled by a metronome at about 70 chews/ min.⁽⁹⁾ Stimulants like citric acid are applied on either side of dorsal surface of tongue.^(7,8) Interestingly, pharmacological and electrical stimulants have also been used as therapeutic aids in the management of patients with salivary gland hypo function.⁽⁹⁾ Thus, the study of unstimulated salivary secretion is an accurate method to analyse salivary gland status, while stimulated saliva is useful for the study of the functional reserve.⁽¹⁰⁾

Methods for collection of saliva per se:

Whole Saliva: The authors have personally attempted various methods of collection whole saliva, both in stimulated and unstimulated conditions. Whole salivary collection is easy and non- invasive. It has been found to be superior and clinically more relevant in the assessment of overall salivary gland dysfunction.⁽⁹⁾ But, individual gland secretions are considered to be superior to whole saliva for many compositional analytes, because whole saliva contains non – salivary elements such as desquamated epithelial cells, bacteria, GCF and leukocytes.

The methods available presently for collection of whole saliva include draining, spitting, suction and swab method. Shannon has compared flow rates in a group of subjects in different body positions. He reported higher flow rate values in the standing position and lower values in the lying position as compared with the flow rate in the sitting position.⁽⁹⁾ Thus, it is ideal to collect saliva, while the subject is sitting upright with the head slightly tilted forward and the eyes open.⁽¹¹⁾ **Draining Method:** The subject is made to sit quietly with the head bent down and the mouth open to allow the saliva to drip passively from the lower lip into the graduated sterile

tubes. (Fig. 1) Saliva collected by draining is without any stimulation and is more reliable.



Fig. 1: Draining Method

Spitting Method: Saliva is allowed to accumulate in the floor of the mouth and the subject spits out it into the pre-weighed or graduated test tubes. (Fig. 2) The advantage of this method is that it can be used when the flow rate is very low and where evaporation of saliva has to be minimised. The disadvantage is that it might have some stimulatory effect, and hence cannot be used for unstimulated saliva collection.^(5,8)



Fig. 2: Spitting Method

Suction Method: Saliva is allowed to accumulate in the floor of the mouth and aspirated continuously using micropipettes, syringes, saliva ejector or an aspirator.⁽⁵⁾

Swabbing Method: It is performed by introducing a synthetic gauze sponge, pre-weighed swab or cotton pad into the mouth, at the orifices of major salivary glands. The subjects are asked to chew such that the sponge gets soaked within the saliva. Saliva soaked sponge is removed and placed in a sterile test tubes. Though this method is less reliable, it helps in the assessment of the level of oral dryness. It is mainly used in the monitoring of drugs, hormones or steroids.⁽⁵⁾

Gland Specific / Glandular Saliva

Glandular saliva is collected from a specific site of the oral cavity. It includes parotid saliva, submandibular/sublingual saliva and secretions from minor salivary glands.⁽⁹⁾

1. **Parotid saliva:** The parotid duct opening is located on the buccal vestibule, opposite to the first and second molars. Unstimulated parotid salivary flow is very low or even absent; hence it is collected under stimulation. Citric acid solution (2-4% weight/ volume) is used for stimulation. Parotid saliva is collected using a cannula or Lashley cup or modified Carlson Crittenden device. (Fig. 3) The device has an outer and inner chamber. The inner chamber is attached to a plastic tubing. The outer chamber is attached to a rubber bulb or a suction device via plastic tubing and the cup is placed over the ductal opening. The suction device used may be dental suction unit, oil-free portable vacuum pump or laboratory suction bulb.⁽⁹⁾ The authors have found that an average of 1-1.5 ml of parotid saliva can be collected in 10-15 minutes. It must be noted that the protein and organic material concentration of parotid saliva is twice as high as submandibular and sublingual saliva.⁽¹²⁾



Fig. 3: Collection of saliva from parotid gland using Carlson Crittenden cups

The disadvantages of this procedure are that it is complex, slow and invasive. It requires skilled personnel and expertise, since this is accomplished either by cannulation of the glandular ducts or by the application of specific collecting devices to the opening of ducts. Parotid salivary flow rate has been shown to reach its peak value, seasonally during the winter and shows circadian variation with peaks in the afternoons. The time of the day and the year should be thus standardised for saliva collection, as it is a potential factor influencing the flow rates in long-term salivary studies.⁽⁹⁾

2. **Submandibular/ sublingual saliva:** This glandular saliva can be collected by cannulation, segregator

methods and suction methods. The simplest method for collection of submandibular saliva is the suction method.⁽¹³⁾

- **Suction method:** It is collected by blocking the Stensen's duct using cotton roll or Lashley cup. Then, the saliva which gets accumulated in the floor of the mouth is aspirated using a syringe or micropipette or with gentle suction
 - **Cannulation:** Tapered polyethylene tubing can be used for cannulation of the Wharton's duct. The thin duct which is prone to rupture poses to be the biggest disadvantage of this method.
 - **Segregator method:** An apparatus capable of collecting submandibular and sublingual saliva with masticatory as well as gustatory stimuli has been fabricated and reported. The collector is placed on the lower jaw and the polyethylene tube connects the chamber to the collecting tube. The appliance should possess adequate peripheral sealing and proper retention to minimise the intermixing of submandibular and sublingual saliva and further eliminate the contamination by parotid saliva. The central chamber collects the sub-mandibular saliva, while the two lateral chambers collect sub-lingual saliva. The procedure is time consuming because the device has to be fabricated and adjusted on an individual basis.^(5,14)
3. **Collection of saliva from minor glands:** Saliva from minor salivary glands includes palatine saliva, buccal and labial saliva. Labial and buccal saliva can be collected using the periopaper / sialopaper absorbent method. (Fig. 4) The quantity of saliva can be determined by periotron.⁽⁹⁾ Palatine saliva is collected using filter paper (periopaper) or pipette method or by a collecting prosthesis.



Fig. 4: Sialopaper, used for saliva collection from minor salivary gland

Armamentarium required for collection of whole and gland specific saliva

The authors have tabulated the armamentarium required for collection of saliva for the reader's comfort. (Table 1, Fig. 5 a-d)



Fig. 5a: Graduated sterile tubes with conical end



Fig. 5b: Automated micropipette for aspirating saliva



Fig. 5c: Dental suction tubes



Fig. 5d: Plastic conical tubes used for collecting saliva

Table 1: Armamentarium required for collection of whole and gland specific saliva

Whole saliva	<ol style="list-style-type: none"> 1. 50 mL sterile tube and paper/styrofoam cup 2. Crushed ice and container 3. Distilled water
Parotid saliva	<ol style="list-style-type: none"> 1. Lashley cup fitted with appropriate polyvinyl chlorate tubing. This device requires suction, which can be provided by a dental unit suction laboratory suction bulb or oil-free portable vaccum pump. 2. Low-affinity conical plastic collection tubes on ice 3. Approximately 5 mL of sterile 2% w/v aqueous citric acid solution, stored at room temperature.
Submandibular /Sublingual secretion	<ol style="list-style-type: none"> 1. Submandibular and sublingual saliva collector fitted with a sterile 100 µL pipette tip and a low-affinity plastic conical collection tube. This device requires suction, which can be provided by a dental unit suction or oil-free portable vacuum pump 2. Distilled water 3. Sterile cotton sponges, dental mirror and forceps 4. Approximately 5 mL of sterile 2% w/v aqueous citric acid solution

Guidelines for collection of saliva

The subject should be made to sit comfortably in a calm and isolated room. He / she should rinse the mouth thoroughly using distilled water or deionized water to remove any food de-bris. The subjects are then asked to

spit out the saliva that has been collected in the initial 30 seconds. They are also trained to collect the saliva in the floor of the mouth for whole saliva collection. High quality polypropylene tubes or vials should be used for collection.⁽¹⁵⁾ Initial two minutes of parotid saliva

secretion and any type of stimulated saliva should be discarded, to avoid salivary diluting effect.⁽²⁾

Collection should be made at a standard time, preferably between 8 to 11 am. The subject should preferably be in the fasting state or two hrs after breakfast.⁽¹⁰⁾ The participants should not brush their teeth for a duration of 45 min prior to the sample collection. Denture wearers should remove their dentures prior to saliva collection.⁽¹⁵⁾ Dental work or oral examination should not be performed within 24 hrs prior to the sample collection.⁽¹⁶⁾ Participants should be screened for any oral health problems or injuries. Visibly contaminated samples with blood should be discarded. The subjects should avoid smoking for at least two hours prior to saliva collection.

Recent techniques of collection of saliva

Newer techniques are mostly modifications of the expectoration methods. A few of them include Oragene, Saligene, Oracol and Verofy. Oragene is the most sophisticated technique, where preservatives are added to protect the sample integrity. Using this method, 0.5ml of saliva can be collected and used in genetic analysis/testing.^(16,17) Saligene is an alternative technique which uses collection tubes into which saliva is expectorated for a predetermined volume, following which a plunger is placed to cap the tube.

Oracol, on the other hand, is based on saliva collection through an absorbent foam swab, which picks up 1 mL of saliva. Once the saliva has been taken, it is secure within the container. A micro tube is incorporated within the device, so that the saliva is centrifuged directly into the final container. This reduces the risk of aerosol contamination. This is particularly beneficial when used in the field, where laboratory facilities are not available.^(16,17) Oracol is used in salivary diagnosis of measles, human immunodeficiency virus (HIV), hepatitis A and B, mumps and rubella. Verofy is a unique method which utilises high quality immunochromatographic strips for delivery of immediate results.⁽¹⁶⁾

With more sensitive and standardised techniques and standard reference values, salivary diagnostics is gaining promise as a technique of choice presently. Recent advances in methods of salivary collection and use of multiplex assays that are versatile, sensitive, specific, rapid, cost effective for broad implementation in diagnostic programs makes the future of salivary diagnostics to appear very promising.

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