

## Saliva – a charismatic fluid

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**Abstract**

Oral fluid is often called the ‘mirror of the body’. The noninvasive nature of salivary testing has made it an attractive and effective alternative to blood and urine. Use of saliva has become more extensive in recent years, particularly in relation to estimation of systemic levels of lipid-soluble drugs and hormones. Furthermore, saliva may provide a cost-effective approach for the screening of large populations. Gland-specific saliva can be used for diagnosis of pathology specific to one of the major salivary glands. Saliva as a whole, however, is most frequently used for diagnosis of systemic diseases, since it is readily collected and contains serum constituents. As a diagnostic fluid, saliva offers distinctive advantages over serum because individuals with modest training can collect it non-invasively. This review presents the translational value of saliva as a credible clinical diagnostic fluid and the scientific rationale for such use.

**Key words:** Saliva, Salivary glands**Introduction**

In humans, oral fluid mainly originates from three pairs of major salivary glands namely parotid, sublingual and submandibular and minor salivary glands. Saliva is a complex secretion. 93% by volume is secreted by the major salivary glands and the remaining 7% by the minor glands.

Daily secretion is 500 -700 ml and the average volume in the mouth is 1.1 ml. At rest, secretion is 0.25 to 0.35 ml/min and is mostly produced by the submandibular and sublingual glands. Several physiological and pathological conditions can modify salivary production quantitatively and qualitatively, e.g., smell and taste stimulation, chewing, psychological and hormonal status, drugs, age, hereditary influences, oral hygiene<sup>1</sup> and physical exercise.<sup>2,3</sup> Sensory, electrical or mechanical stimuli can raise the secretion rate to 1.5 ml/min. The greatest volume of saliva is produced before, during and after meals, reaching its maximum peak at around 12 a.m., and falls considerably at night, while sleeping.<sup>4</sup>

99% is water and the other 1% is composed of organic and inorganic molecules. While the quantity of saliva is important, so is its quality, as each of its components performs a series of specific functions (summarized in table 1).<sup>5</sup>

**Table 1**

Components	Functions
Mucin, proline-rich glycoproteins, water	Lubrication
Lysozyme, lactoferrin, lactoperoxidases, mucins, cystins, histatins, immunoglobulins, proline-rich glycoproteins, IgA	Antimicrobial action
Mucins, electrolytes, water	Maintaining mucosa

	integrity
Water	Cleansing
Bicarbonate, phosphate, calcium, staterin, proline-rich anionic proteins, fluoride	Buffer capacity and remineralisation
Water, mucins	Preparing food for swallowing
Amylase, lipase, ribonucleases, proteases, water, mucins	Digestion
Water, gustin	Taste
Water, mucin	Phonation

Various salivary investigations have been proposed for diagnosis and assessment of systemic disease. Thus its importance as a valuable diagnostic tool cannot be denied. Hence, it is imperative to understand the usage of saliva in detection of various diseases and pathologies.

**Saliva collection**

Saliva has a simple and noninvasive collection method.

Saliva produced by a single salivary gland can be collected by cannulation of a single salivary duct,<sup>6</sup> or by a metal or acrylic cup placed over the Stenson's duct for the collection of pure parotid saliva.<sup>7</sup> Crevicular fluid can be collected by inserting an appropriate device directly<sup>8</sup> such as filter paper, a commercial micropipette or a thin tube. Saliva from submandibular glands can be collected by placing the tip of a collection device at the orifice of the Wharton's duct, after placing sterile cotton sponges in the floor of the mouth and over the buccal mucosal areas to occlude the parotid and sublingual ducts.<sup>7</sup> Unstimulated whole saliva can be collected by passive drooling (no oral movements): allowing saliva

to drain off the lower lip into a plastic vial.<sup>9,10,11</sup> Stimulated saliva may be obtained using non absorbing methods such as chewing a piece of paraffin wax of standardized size.<sup>12,13</sup> and chewing neutral gum base.<sup>10</sup> Chewing parafilm or rubber bands can also be helpful in stimulating saliva. Keeping in mouth powdered drink crystals<sup>14</sup> or other foodstuff containing citric acid.<sup>15</sup> The most commonly used devices are sterile cotton dental rolls as Salivette (Sarsted, Newton, NC). However, Cotton wool sampling may induce variations in salivary immunoassays: testosterone, DHEA, estradiol, 17-OH hydroxyprogesterone.<sup>16</sup> To avoid this type of analyte interference, non-cotton based sampling are available such as:

1. Polystyrene foam swabs.<sup>11</sup>
2. Rayon balls, such as Orapette (Trinity Biotech, Dublin, Ireland).<sup>16</sup>
3. Polyester Salivette (Sarsted, Newton, NC).<sup>17</sup>

Saliva investigation for diagnostic purposes has been proposed in the following:

1. **Caries:** The use of saliva in diagnosing caries risk is well-known, particularly in monitoring chemical treatments to control the disease,<sup>18</sup> owing to the possibility of detecting the presence of *S. mutans* and *Lactobacillus* spp, as well as lactic acid, which causes the sub-surface demineralisation that causes the onset of the caries lesion.<sup>19</sup>
2. **Periodontitis:** Higher enzyme activities were found in adult periodontitis patients compared to the healthy controls for alkaline phosphatase, esterase,  $\beta$ -glucuronidase,  $\beta$ -glycosidase, and other amino peptidases. Saliva from patients with localized juvenile periodontitis contained the highest levels of butyrate esterase and cysteine amino peptidase.<sup>20</sup>
3. **Infections:** Correlation between salivary and serum antibodies has previously been reported for HIV (human immunodeficiency virus). Salivary IgA levels to HIV decline as infected patients become symptomatic. It was suggested that detection of IgA antibody to HIV in saliva may, therefore, be a prognostic indicator for the progression of HIV infection.<sup>21</sup>

Correlation between salivary and serum IgG levels is also present in HCV antibodies,<sup>22</sup> HAV (hepatitis A virus),<sup>23</sup> EBV (Epstein Barr virus),<sup>24</sup> CMV (Cytomegalo virus) and rubella virus. Salivary antibodies have also been reported after immunization against poliovirus, rotavirus and HAV.<sup>22</sup>

*H. pylori* exists in higher prevalence in saliva than in faeces, and the oral-oral route may be an important means of transmission of this infection in developed countries.<sup>25</sup>

4. **Cancer:** c-erbB-2 soluble fragments and 15-3 cancer antigen in breast cancer research have been demonstrated in saliva.<sup>26</sup> In ovarian cancer too, the CA 125 marker can be detected in the saliva with

greater specificity and less sensitivity than in serum.<sup>27</sup> The presence of protein p53 antibodies in patients with oral squamous cell carcinoma, or high levels of defensin-1 positively correlated with the serum levels. Higher concentrations of salivary defensin-1 were detected in patients with oral SCC in comparison to healthy controls.<sup>28</sup>

5. **Drugs:** Saliva may be used for monitoring patient compliance with psychiatric medications.<sup>29</sup> Many substances which are commonly abused can be detected in human saliva. Including alcohol, amphetamines, barbiturates, benzodiazepines, cocaine, lysergic acid diethylamide (LSD), opioids, phencyclidine and cotinine for tobacco smoke.<sup>30</sup>

A significant correlation between salivary and serum alcohol levels was reported.<sup>31</sup>

Salivary ethanol concentration may be used as an index of the blood ethanol concentration, provided that the salivary sample is obtained at least 20 min following ingestion. This will allow for absorption and distribution of alcohol, and prevent a falsely elevated reading due to the oral route of consumption.<sup>32</sup>

The presence of thiocyanate in the saliva is an excellent indicator of active or passive smoking. As determined by Maliszewski and Bass (1955), thiocyanate concentrations are higher in the saliva of smokers than in that of non-smokers. This observation has proved to be of value in the confirmation or rejection of self-reports of cigarette usage among children and adolescents (e.g. by Luepker et al., 1981). Passive smoke exposure leads to elevation of IL-1b, albumin and aspartate aminotransferase (AST) levels in saliva.<sup>33</sup> Salivary cotinine levels were found to be indicative of active and passive smoking.<sup>34,35</sup> Salivary thiocyanate was also found to be an indicator of cigarette smoking;<sup>36</sup> however, cotinine levels are considered the most reliable marker.<sup>37</sup> Other drugs such as cocaine or opiates can also be detected in saliva.<sup>38</sup>

6. **Hormones:** Detection in the saliva of certain hormones such as cortisol, aldosterone, testosterone, estradiol or insulin is highly correlated with concentrations of the same in serum. Generally speaking, it is the liposoluble hormones with lower molecular weights that can be detected most reliably in the saliva, whereas protein-bound hormones will not be found as there is no active transport into the saliva.<sup>39</sup> Putignano et al. proposed midnight salivary cortisol measurement as a screening procedure for patients with suspected Cushing's syndrome.<sup>40</sup> Abnormal salivary diurnal cortisol rhythms have been shown to be predictive of disease progression in metastatic breast cancer patients.<sup>41</sup>

7. **Hereditary disease:** Cystic fibrosis (CF) is a genetically transmitted disease of children and young adults which is considered a generalized exocrinopathy. Most studies agree that saliva of CF patients contains increased calcium levels.<sup>42,43,44</sup>

Elevated levels of calcium and proteins in submandibular saliva from CF patients were found, and resulted in a calcium-protein aggregation which caused turbidity of saliva.<sup>45</sup>

### Role of saliva in orthodontics

At low load levels saliva acts as a lubricant, but at high loads saliva may increase friction if it's forced out from the contacts between the brackets and the arch wire.<sup>46</sup>

A clinical study was carried out to determine the acceptability of a sugar free, low tack chewing gum by orthodontic patients. It was concluded that low tack sugar free chewing gums can be used by orthodontic patients to increase saliva flow with the potential to remineralise and help reduce white spot lesion formation.<sup>47</sup>

A study was conducted with a purpose to find out whether composition of salivary pellicles that form on surface of orthodontic materials vary qualitatively in respect to stainless steel, elastomeric ligature ring, bracket bonding resin. It was concluded that least amount of salivary pellicle which was cariogenic was found on stainless resin, followed by adhesive resin, highest amount of cariogenic pellicle was found on elastomers.<sup>48</sup>

### Saliva and corrosion

Saliva acts as an electrolyte and hence aids in causing corrosion of metal components of fixed orthodontic appliances. When metal components of orthodontic appliances are in contact with an electrolyte such as saliva, metals corrode by a complex electrochemical process of oxidation and dissolution known as galvanic corrosion. The generation of an electric cell is simple when different metals are involved, but it can also occur within a single metal.

In saliva samples, nickel and chromium reached their highest levels in the first month and decreased to their initial level in the rest of the groups. It can be concluded that fixed orthodontic appliance releases measurable amount of nickel and chromium when placed in the mouth. Most metals used in oral cavity can be expected to undergo this type of corrosion.<sup>49</sup>

### Saliva and demineralization (caries)

The pH of saliva acts as a deciding factor, be it demineralization and induction of caries or remineralization. At pH value of 6.8 to 6.0 hydrogen ions reacts with phosphate ions in saliva and plaque. At pH value of 5.5 to 5.0 demineralization occurs wherein hydroxyapatite dissolves but fluorapatite forms in the presence of fluoride. At pH of 4.5 to 3.5 (critical pH) both fluorapatite and hydroxyapatite dissolves. On the contrary if pH rises to 5.5 from the critical pH and if H<sup>+</sup> ion are not exhausted remineralization occurs and fluorapatite forms.

### Conclusion

With advances in microbiology, immunology and biochemistry, salivary testing in clinical and research settings is rapidly proving to be a practical and reliable means of recognizing oral signs of systemic illness and exposure to risk factors. Since it is non-invasive, its attraction for diagnosis is increasing especially in children. Saliva is particularly useful for qualitative (detection of the presence or absence of a marker) rather than quantitative diagnosis, which makes it an important means for the detection of viral infection (especially HIV due to the non-invasive method of collection), past exposure and immunity, and the detection of illicit drug use. Saliva is also useful for the monitoring of hormone levels, especially steroids, and facilitates repeated sampling in short time intervals, which may be particularly important for hormone monitoring and avoiding compliance problems. Science, is rapidly closing the gap between saliva and other diagnostic biomedica (blood, urine, cerebral spinal fluid, tear, nipple aspirate, faecal matter). Scientific data to benchmark the diagnostic value of saliva against other biomedica will be necessary to assess the disease discriminatory value of saliva.

The day is near when saliva will be considered a diagnostically diverse and charismatic fluid. Use of saliva as a marker of various systemic diseases makes it a promising path in the field of diagnosis.

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