



## Review

## Saliva: reflection of the body

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## ABSTRACT

Saliva has become an important resource for evaluating physiological and pathological conditions in humans. The use of saliva has many advantages, including the simple and non-invasive method of collection and its easy, low-cost storage. With the addition of modern techniques and chemical instrumentation equipment, there has been an increase in its use for laboratory investigations, applicable for basic and clinical analyses in the fields of medicine and dentistry. The value of these methods for the diagnosis of oral and systemic diseases has been the subject of study by several researchers with the aim of increasing its use alongside complementary exams.

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## 1. Introduction

Saliva is an aqueous fluid found in the oral cavity, composed of a complex mixture of secretory products (organic and inorganic products) from the salivary glands and other substances coming from the oropharynx, upper airway, gastrointestinal reflux, gingival sulcus fluid, food deposits, and blood-derived compounds.<sup>1,2</sup>

Saliva is one of the most complex, versatile, and important body fluids, supplying a large range of physiological needs. In the digestive tract, saliva plays an important role in esophageal physiology, the digestive process, and gastric cell protection. In the oral cavity, saliva takes part in mastication, speech, deglutition, gustatory sensitivity, tissue lubrication, mucosal protection against invasion, antibacterial, antifungal, and antiviral activity, post-eruptive maturation, ionic balance regulation at enamel remineralization, deposition of acquired enamel pellicle, and acid diffusion limitation.<sup>3–5</sup>

Water is the greatest component of saliva, representing 99% of its composition. The solid components, organic and inorganic molecules, are found dissolved in the aqueous component and vary widely from one individual to another, and even vary in the same

individual several times during the day. The inorganic part is composed of weak and strong ions, with the most important being Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>−</sup>, Ca<sup>2+</sup>, HCO<sub>3</sub><sup>−</sup>, Mg<sup>2+</sup>, and NH<sub>3</sub>. The organic part contains components such as body secretion products (urea, uric acid and creatinine), putrefaction products (putrescine, cadaverine; lipids such as cholesterol and fatty acids), and more than 400 types of protein. The most relevant proteins have a glandular origin (α-amylase, histatins, cystatins, lactoferrins, lysozymes, mucins, and proline-rich proteins (PRPs)) or are plasma-derived (albumin, secretory immunoglobulin A (sIgA), transferrin).<sup>6</sup>

Salivary analysis has become an important resource for the evaluation of salivary conditions with physiologic and pathologic implications and is a useful tool for disease diagnosis, mainly due to its origin, composition, functions, and interactions with other organ systems. Additionally, it has a simple and non-invasive collection method, is easy to store, and is inexpensive when compared to blood collection. With the addition of modern techniques and chemical instrumentation equipment, there has recently been an observable increase in its use for laboratory investigations, applicable for basic and clinical purposes in dentistry and other medical areas. The value of saliva as a diagnostic tool for oral and systemic diseases has been an area of study for many researchers with the aim of increasing its use as a possible complementary exam.<sup>7–10</sup>

Recently, an increasing appreciation of the use of saliva as a mirror that reflects the normal internal characteristics and disease

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state of an individual has developed.<sup>7</sup> The possible use of saliva as a specimen for diagnosis is due to its exchange with substances that compose the plasmatic liquid. This occurs due to the presence of a thin layer of epithelial cells separating the salivary ducts from the systemic circulation, making it possible for substances to be transferred to the saliva through active carriage, diffusion through the membrane (ultrafiltration), or through passive diffusion via a concentration gradient.<sup>11</sup>

The ability to use saliva to monitor an individual's health and disease state is a highly desirable objective for healthcare research and promotion. Recent scientific and technological advances have produced continued improvements in many areas, such as salivary component determination, comparative samples attainment, and an increase in the specificity and sensitivity of procedures. These advances point to a new age in which molecular diagnosis in the oral cavity will be very important.<sup>12</sup> The present paper examines studies in the scientific literature involving saliva as a diagnostic tool.

## 2. Infectious diseases

Detecting antibodies, produced due to an immunological response to infection, is the fundamental principle of many diagnostic tests in virology, and these molecules can be found in saliva originating from the salivary glands and in blood serum. There is a preponderance of sIgA, derived from the plasmacytes in the salivary glands, and this represents the main mechanism of the saliva-specific immunologic response.<sup>13</sup> In contrast, immunoglobulins M (IgM) and G (IgG) are also derived from serum through ultrafiltration and form part of the gingival crevicular fluid becoming a component of the whole saliva. These antibodies act against microorganisms and its components can be detected in the saliva, thus representing an important resource for the diagnosis of acute viral and congenital infections.<sup>14</sup>

Dental caries is an infectious disease characterized by localized destruction of tooth tissue due to the action of bacteria; this affects a large number of individuals in the world. In the last few decades, there has been great interest in the utilization of saliva for bacteriological tests that give an indication of caries risk.<sup>15,16</sup> These tests are based on the identification of *Lactobacillus* species and mutans streptococci and their quantification in saliva, as these bacteria play a significant role in caries development. Identifying those individuals most susceptible to the disease through saliva tests could strengthen preventive measures for this disease.<sup>17</sup> Other infectious diseases of the oral cavity can be diagnosed in this way, such as candidiasis through the presence of *Candida spp* in the saliva.<sup>18</sup>

The use of saliva as a diagnostic and monitoring method for periodontal disease has been increasingly studied.<sup>19</sup> This pathological disease is caused by specific microbial groups that attack supportive and protective periodontal tissues; consequently, there is tissue collagen degeneration resulting in the progressive destruction of periodontal ligament and alveolar bone.<sup>20</sup> Many studies have already correlated an increase in immunoglobulin levels with the presence of pathogens in patients with periodontal disease.<sup>21,22</sup> Besides these molecules, the analysis also detects enzymes, gingival fluid components, and bacterial components. Todorovic et al.<sup>23</sup> analyzed the saliva of patients who had periodontitis and demonstrated significant increases in enzyme activity (aspartate and alanine aminotransferases, lactate dehydrogenase, creatine kinase, alkaline and acidic phosphatases, and  $\gamma$ -glutamyl transferase) in association with cell injury and tissue cell death, concluding that salivary enzyme activity, used as biochemical markers, may be useful in the diagnosis, prognosis, and monitoring of periodontal disease. Takane et al.<sup>24</sup> analyzed the molecule 8-hydroxydeoxyguanine (8-OHdG) with regards to

periodontal tissue damage in saliva samples from 78 patients with this disease, before and after treatment, and concluded that this marker indicated the actual periodontal condition of the patients studied.

Studies that use saliva for the diagnosis of HIV using specific antibodies as biological markers<sup>25,26</sup> have been demonstrated to be successful and reproducible. This method made the first quick test for the detection of HIV-1 infection possible, a test that is presently used in investigations and has a high sensitivity and specificity, at 99.4%.<sup>25</sup> In 2007, Cuban scientists compared HIV-1 diagnosis using saliva with a serological exam in 125 seropositive patients. The sensitivity and specificity of the saliva was 100%, demonstrating the usefulness of this tool as an alternative for the confirmation of antibodies against HIV-1.<sup>27</sup>

The US Centers for Disease Control and Prevention (CDC) has been using a saliva-based test for the detection of HIV that provides results in only 20 minutes. This speedy test is extremely important because it has been observed that there is a higher probability of individuals taking the test if the exam is simple and gives a rapid result. Studies have demonstrated that when conventional tests are used, which require the patient to return in two weeks to get the result, approximately one third of the patients never return. In Brazil, Anvisa (the national agency of sanitary vigilance) has recently approved an oral test for HIV detection using saliva that is capable of detecting HIV types 1 and 2 with 99% accuracy. This test will be used in health centers, hospitals, clinics, and laboratories. Rapid detection also helps in disease prevention; once most patients become aware that they are infected with HIV, they take care to avoid transmission and obtain healthcare that can lengthen their lives and improve their quality of life.<sup>28</sup> Through interviews with injection drugs users, a population especially vulnerable to HIV infection, it was verified that these users were receptive to the possibility of taking the quick saliva test and considered this method the best option when compared to the available conventional tests. Additionally, the fact that this method does not require venous blood collection was considered an advantage, especially for those who have veins damaged by the injection of drugs and for those who report a fear of needles or blood collection.<sup>29</sup>

Hepatitis, a disease that causes liver inflammation that has a viral etiology, can be diagnosed through the saliva. Van der Eijk et al.<sup>30</sup> demonstrated the first precise quantitative measurement of hepatitis B virus (HBV) DNA levels in the saliva and compared the results with those from blood. The results of this study showed that saliva could be a source of HBV. Moreover, in 2000, Zhevachevsky et al.<sup>31</sup> presented the same observation while analyzing the diagnostic value of saliva to detect HBV in patients, proving its role as an investigative element. A method developed by a Brazilian research institute (FIOCRUZ–Oswaldo Cruz Foundation) identifies hepatitis A cases even in infected individuals who are in the immunological window period. This is possible due to the utilization of PCR on saliva samples. The efficacy of this method was proven by demonstrating that saliva has a higher virus level than the blood samples; the virus frequency in saliva samples was 37%, while the blood analysis showed a 32% frequency. This test has proved to be very important for early viral detection and the control of possible epidemic outbreaks.<sup>32</sup>

The use of saliva as a diagnostic tool for *Helicobacter pylori* infection (a species of bacteria that infects the mucosal surface of the stomach and may cause peptic ulcers, gastritis, and cancer) is an attractive option for epidemiologic studies in children when the non-invasive nature of the test is considered. It is normal practice to use gastric biopsy samples in *H. pylori* identification tests; however, these invasive methods have high costs and are more difficult to perform. In this case, PCR technology has proved to be highly sensitive and specific for detecting *H. pylori* DNA in the mouth using biological markers found in saliva.<sup>33</sup>

Saliva samples can also be used for the laboratory diagnosis of rubella through the detection of immunoglobulin M (IgM). In 2000, Oliveira et al.<sup>34</sup> showed that IgM antibodies specific against rubella were found in 84.4% of salivary samples with a test specificity of 96%. Nokes et al.<sup>35</sup> analyzed blood and saliva samples of 853 individuals of all ages from a rural community in Ethiopia to detect rubella-specific antibodies; the saliva test showed a sensitivity of 79% and specificity of 90% higher than the blood test. These results indicate that using saliva can be a valid alternative for obtaining clinical specimens for the investigation of recent cases of this disease, especially for epidemiological surveillance and virus control.

Dengue is a viral disease transmitted by the mosquito *Aedes aegypti*. Primary infection can lead to a self-limiting febrile disease, while a secondary infection can cause serious complications such as hemorrhagic fever or dengue shock syndrome.<sup>36</sup> Specific tests for salivary antibody levels for dengue (IgM and IgG) showed 92% sensitivity and 100% specificity in the diagnosis of primary and secondary infections. Additionally, IgG salivary levels are useful for the differentiation between primary and secondary infections.<sup>37</sup> Research conducted in India on 80 patients suspected to have dengue and 25 control patients showed a good correlation between the diagnostics using saliva and the conventional test (through venous blood use), concluding that saliva has effective markers for dengue diagnosis.<sup>38</sup> Analyzing the kinetics of three serological markers (IgM, IgA, and IgG) in serum, saliva, and urine samples from adult patients with primary or secondary dengue infection, Vazquez et al. concluded that the specific IgE could play a role as a serological marker in secondary infections.<sup>39</sup>

### 3. Malignant neoplasia diseases

Human DNA has been shown to be useful as a biological marker and its utilization has been studied for the detection of some types of neoplasias. Most of the specimens used for this type of investigation are collected through invasive biopsies and, generally, when analysis is possible, the tumor is already settled or even in a metastatic state. This suggests a need for the development of new diagnosis tools that possibly lead to the early detection of the disease. The identification of molecular markers in body fluids that predict cancer development in its primary or pre-cancerous phases could help establish this new tool.<sup>40</sup> A recent report suggests that head and neck carcinomas can be detected utilizing DNA derived from the exfoliation of oral mucosa cells collected in saliva.<sup>41</sup> Franzmann et al.<sup>42</sup> evaluated the use of CD44 protein in saliva as a potential molecular marker for head and neck cancer and concluded that the test can be effective for the detection of this kind of cancer for all stages.<sup>8</sup> Li et al.<sup>40</sup> showed that malignant tumors located in the head and neck can be diagnosed through saliva with 91% precision, important for early diagnosis and increasing the possibility of successful treatment. Breast cancer diagnosis through salivary samples has already been studied and Streckfus et al.<sup>43</sup>, analyzing the salivary samples of 30 patients, identified 49 proteins that differentiate healthy patients from those with a breast cancer diagnosis. Research showed that the presence of this kind of tumor produces changes in the amount and the characteristics of proteins found in the salivary glands. Salivary protein analysis also distinguished if breast tumors were malignant or not.

### 4. Forensic analysis

The capacity of detecting human DNA in saliva has also been useful in forensics. Saliva can be found in many areas inside a crime scene such as in bites marks left in objects or victims of violent crimes, cigars, postage stamps, envelopes, and other objects.<sup>44</sup> It

has been shown that saliva could be potentially recovered in such cases. A study conducted by Anzai-Kanto et al.<sup>45</sup> obtained the saliva of volunteers from their skin for the extraction and identification of DNA through PCR, for posterior evaluation of its utilization and its contribution to forensic dentistry. The results indicated that standardized procedures used for collect and extraction of salivary DNA can be used as a method to recover DNA in forensic cases, since there were enough quantities for analysis. This would allow such tests to be incorporated into the evidence of a criminal investigation, giving it great discriminatory capacity.

### 5. Investigation of pharmaceutical and illicit drugs

The diffusion of substances in body liquids and saliva allows for their use to monitor pharmaceutical drug levels (lithium, carbamacepine, barbiturates, benzodiazepines, phenytoin, theophylline, and cyclosporin), legal drug levels (alcohol and tobacco), and illicit drug levels (marijuana, cocaine, and amphetamines).<sup>46</sup>

Lithium, a drug used for treating bipolar disorders with weak therapeutic registers, demands a constant monitoring of its concentrations in the blood serum to obtain a better therapeutic effect and to reduce adverse effects, and its monitoring through saliva would be a useful alternative.<sup>47</sup> Besides being measured in the serum, lithium can also be measured in saliva and erythrocytes. In these cases, its numeric representation is done in relation to the concentration found in the serum or blood, which can vary from 3 to 13 when the concentrations are higher in saliva.<sup>48</sup>

In the case of licit and illicit drugs, it is known that the indiscriminate use of these substances in different society levels is a worldwide phenomenon that has caused deep concern among specialists. Differences concerning the rate of use and kinds of drug can be observed from country to country. However, the impact on public health, individual safety, and social structure are universally negative. Legal drugs, such as alcohol and tobacco, and illicit drugs, such as marijuana, cocaine, and amphetamines are used by millions of people all over the world, generally with serious consequences for the user and for the society. Fatalities occur not only due to acute or chronic intoxication, but also due to behavior and psychomotor alterations that such substances can cause in users. In the last few years, an increasing worldwide interest has been observed related to the use of saliva to monitor the use of drugs by drivers. The advantage in the use of saliva lies in the fact that this oral fluid can be easily collected in a non-invasive way and under direct observation, making the possibility of sample adulteration by the donor difficult. Also, using saliva as a means of detection could indicate, in a few minutes, if a driver was driving under the influence of drugs.<sup>49</sup>

### 6. Hormonal analysis

The knowledge that there are steroid hormones in saliva that they can be measured has been around for more than 30 years. However, only recently has the technology reached this information, making it possible to exactly determine hormonal levels in saliva. Evaluations can be accomplished in blood samples, but this has its limitations. Most blood hormones (approximately 95%) are limited to specific proteins that carry them in the blood stream but this is only a fraction of the hormones in storage. The other 5% represent free hormones, available to move easily to their target organs and perform their functions. Saliva contains some free hormones that can be easily measured to give an exact view of those readily available in human tissue. The detection of some of these free hormones is important because their variation in saliva can be indicative of cancer progress or the possibility of a disease like Cushing's syndrome, a disease that results from the continuous hypersecretion of endogenous cortisol,

which leads to proximal musculature weakness, osteoporosis, spontaneous ecchymosis and hypocalcemia. Estrogens also can be verified in saliva and the prediction of premature birth can be detected through salivary estradiol measurement, a test approved by the FDA (Food and Drug Administration). An increase of this hormone is also an indication that a woman is in her fertile period and creating a self-test with a commercially distributed device gives her the capacity to monitor her fertile cycle.<sup>50</sup> Additionally, it is known that hormonal changes can lead to systemic disorders such as, for example, the diabetes mellitus.<sup>51–53</sup>

## 7. Autoimmune diseases

Sjögren's Syndrome is a chronic autoimmune disease, characterized by dysfunction in salivary and lachrymal glands, keratoconjunctivitis sicca, xerostomia, in addition to serological abnormalities. Some procedures for the diagnosis of this syndrome include sialography, salivary scintigraphy, biopsies, and serological tests. Although they are useful, these tests are invasive, expensive, and not always conclusive.<sup>54</sup> Researchers have measured specific concentrations of cytokines (group of molecules involved in the transmission of signals among cells during the beginning of the immune response) in the saliva of patients with Sjögren's Syndrome for their eventual use in diagnosis. Interleukins 2 and 6 are found in levels significantly high in individuals that suffer from this disease, thus, alterations in the salivary profiles of these cytokines can be useful in the diagnosis of Sjögren's Syndrome as well as in the control of its progression.<sup>55</sup>

## 8. Cardiovascular diseases

Cardiovascular diseases are a leading cause of death all over the world.<sup>56</sup> Markers found in saliva, such as amylase, have been used for post-operative control of patients who had cardiovascular surgery. A study of Adam et al.<sup>56</sup> showed that low levels of salivary amylase in the pre-operative stage of patients with aorta aneurism is associated with an increase in mortality. Another study, done by Samaranayake<sup>57</sup> in 2007 verified that the alpha amylase salivary activity could be used as a good marker of catecholamines during the evaluation of patients in different stressful situations. These investigations demonstrate the possibility of using saliva to evaluate the general health of an individual. Unfortunately these investigations are only in the initial development phases, needing confirmation of its general utility.

## 9. Discussion

The advantages of using saliva for diagnosis compared to other biological specimens are the easy availability, simple and non-invasive collection that can be done by the individual themselves, and easy, low-cost storage. In addition to these advantages, saliva collection offers a painless alternative, eliminating the stress that the patient can feel,<sup>10</sup> which can be useful for geriatric, pediatric, and obese patients, patients with mental deficiency, prisoners, etc.

One main disadvantage of the utilization of saliva as a diagnostic liquid is the fact that some substances submitted to analysis are found in lower quantities in saliva than in blood. The biochemical concentration of these components in the blood stream is well documented, regulated, and defined by variation scales with reference values. There are straight scales for plasma with little variation.<sup>7</sup> However, with new and highly sensitive techniques, the low levels of molecules analyzed in saliva are not a limitation. Almost any element that can be measured in the blood can be measured in the saliva due to the development of nanotechnology, which has allowed researchers to handle substances at an atomic scale.<sup>43</sup>

The National Institute of Dental and Craniofacial Research (NIDCR) has a program to develop the use of saliva as a diagnostic tool, with the aim to make this a viable technology that could lead to its commercialization. In 2003, NIDCR supported research aiming to identify "salivary DNA," a sort of periodic table of salivary components, through the detailed cataloguing of human salivary proteins in the three main glands. This would be a fundamental resource for the elucidation of disease pathology, in addition to evaluating drug influence on the structure, composition, and secretion of all salivary components.<sup>58</sup>

The use of saliva as a diagnostic tool can become a clinical reality through the establishment of a scientific criteria and making clinical validations necessary to make it a highly exact and usable technology to reach a definitive evaluation.

## 10. Conclusions

Saliva is a body fluid that is of high importance for determining physiological and pathological situations of the human body.

In many situations, saliva already contributes for laboratory investigation, such as infectious disease diagnosis, malignant neoplasias, pharmaceutical and illicit drugs monitoring, autoimmune diseases, hormonal analysis, and assisting forensic medicine.

The advantages of using saliva in laboratory diagnosis are that it is readily available, easy to collect, non-invasive, and has a simple, low-cost storage.

An inconvenience in the use of saliva is the fact that concentrations of its biological markers are found in lower levels in comparison with plasma, and there still are not reference values. The development of better technology (nanotechnology) is improving this situation.

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