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Contributions of salivary tests in dental practice

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Abstract

Over the past two decades, less invasive salivary diagnostic tests have grown dramatically to replace painful and expensive blood sampling. The role of saliva in this growth trend has been enormous, due in part to a growing awareness of the great utility of saliva as a diagnostic tool within clinical settings and more specifically dental offices, as well as the increasing number of publications supporting new and varied applications of saliva.

With this objective in mind, we have conducted a scientific paper on rapid, multiplex and miniaturized analytical tests based on saliva in dental practice and their major contributions to determine non-invasively which pathology, which prognosis and which will be the judicious therapeutic strategy for the management of patients

Keywords: Salivary tests; Dental practice; Saliva in dental practice

1. Introduction

"Saliva" in addition to its numerous known functions in absorption, mastication, digestion and self-cleaning is of increasing interest in the fields of medical analysis as a support for diagnosis, screening, prognosis and evaluation of treatment as an alternative to blood and urine (1).

The richness of its composition could allow the detection of numerous pathologies, via biomarkers related to oral and systemic diseases, in an easier and less expensive way than blood tests (2).

By following the protocol of an initial dental consultation and with the advent of comprehensive patient management, the dentist has become a front-line player in prevention and screening. Continued efforts in this area could lead to the establishment of clinically acceptable salivary tests for the detection and monitoring of various body conditions (3).

2. Properties of salivary tests

2.1. Advantages of salivary tests

The analysis of blood and its components has been the mainstay of laboratory diagnostic procedures for many decades. However, other biological fluids are also frequently used for disease screening, mainly saliva which can offer distinct advantages, (8) such as simple and non-invasive collection method. Sampling is safe for the operator and the patient, and storage is easy and inexpensive. These features allow for the monitoring of several biomarkers in infants, children, elderly and non-collaborative subjects, and in many circumstances where blood and urine samples are not available. Another reason saliva is attractive for diagnostic purposes is its link to traditional biochemical parameters that appear in the circulation in various forms.

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Saliva analyses have been used mainly in dentistry and for studies on various oral diseases to assess the risk of caries and periodontal pathologies, measuring the buffering capacity of saliva and the bacterial content (9).

There are compelling reasons to explore saliva as a diagnostic tool. (10)

- It clearly meets the demands for an inexpensive, non-invasive, and easy-to-use screening method. (11)
- As a diagnostic sample in the clinic, saliva has many advantages in terms of collection, storage, shipping, and sampling All of these processes can be performed very economically compared to serum or urine (11).
- Saliva is also easier to handle in diagnostic procedures than blood because it does not clot, thus reducing the number of manipulations required. (11)
- For healthcare professionals, a saliva test is safer than using serum, which is more likely to expose operators to blood-borne diseases.
- For patients or candidates, the non-invasive collection approach could significantly reduce anxiety and discomfort,
- Collection is generally economical, safe, easy and can be performed without the assistance of health care personnel
- It allows for cost-effective, home-based collection (12)
- It is considered an acceptable and non-invasive process by patients because it is painless (and can be easily collected for patients in the pediatric age range)

These advantages have ensured the widespread use of saliva as a diagnostic tool in clinical practice. The future of this field will depend on further validation of disease-specific biomarkers and their incorporation into a state-of-the-art test that is quantitative, specific, rapid, reliable, sensitive, robust, and cost-effective for broad implementation in diagnostic programs. In the foreseeable future, current research on saliva-based diagnostic methods could revolutionize general and dental health care. (13)

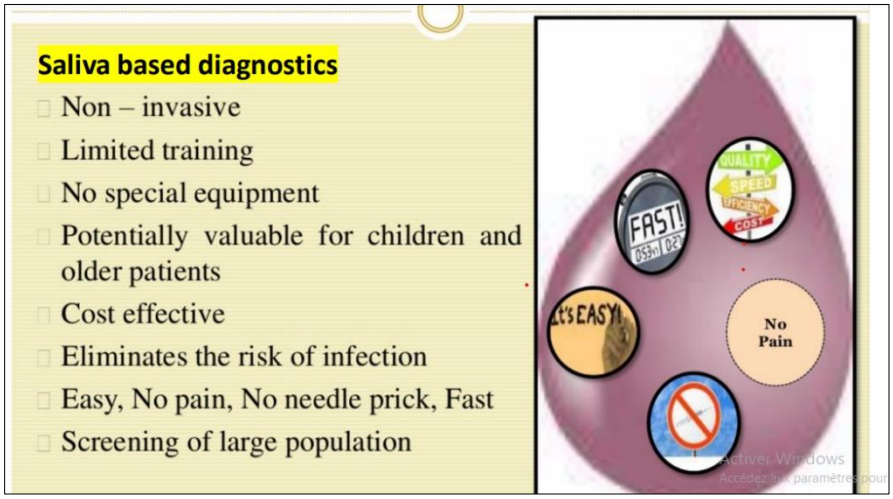


Figure 1 The value of saliva-based testing. (14)

2.2. Limitations of saliva testing

In the last decade, salivary tests are considered a promising modality to provide early and accurate diagnosis, better prognosis and good post-treatment follow-up (3). With the use of new technologies and the in-depth study of salivary composition by advanced methods, oral fluid has far surpassed It has expanded its academic interest and its diagnostic potential and accreditation in the daily practice of the dental physician (11).

Saliva can be considered a mirror of oral and systemic health, but the levels of certain biomolecules are not always consistent with the levels of these markers in serum (15). Salivary composition may vary depending on the method and timing of collection, the technique used, and the degree of stimulation of salivary flow (2). These alterations, combined with changes in pH and variability in local biological fluid flow are factors that influence the expression and release of salivary biomarkers (16). Salivary gland function is furthermore can be affected by a number of systemic disorders, numerous drugs and radiation therapy. In addition, proteolytic enzymes derived from the host and oral microorganisms in whole saliva disrupt the stability and concentration of certain biomarkers. (2)

The performance of salivary tests is inherently limited by the quality of the disease-specific biomarkers, as well as by the nature of the sample with which it is challenged. These biological issues undermine the credibility of the devices and represent barriers to sensitive, specific, and affordable rapid diagnostic salivary tests within dental practices. (4)

Nevertheless, salivary rapid diagnostic tests are based on several core technologies that are subject to a number of potential technical vulnerabilities. These technical complications can render the tests inoperable and less reliable, resulting in decreased test performance in hospital settings. Common technical difficulties include insufficient stability of components under heat stress, poorly characterized affinity reagents, risk of erroneous readings due to subjective interpretation, time dependence of signal development, insensitivity to low disease loads, and unsuitability for pathogen quantification (4).

An additional challenge for the point-of-care rapid test platform is the financial factor: The social value and added benefit to human health by the adoption of salivary diagnostic tests within hospital settings and more specifically dental offices, can only be realized when the cost of the devices is widely affordable by both the dentist and the patient. (5)

To improve health care in organized dentistry, all segments of the dental profession must address the broad applications of salivary testing, including education, reimbursement, and patient acceptance, as well as provide extensive and well-organized educational initiatives to dentists, health care teams, the dental benefits industry, regulatory agencies, and other stakeholders to expand the use of diagnostic devices and ensure better access to health care and early detection of disease within the dental office. (17)

3. Contributions of salivary testing in the dental office

Close monitoring of patients in the dental office via salivary testing is an essential task for the identification of local and general risks. A considerable proportion of the population reported visiting the dentist without consulting their physician. Improved patient health, including quality of life, is associated with dental intervention. (18) In this regard, several studies have shown a positive attitude and wide acceptance of salivary diagnosis in the daily practice of dental health professionals, in order to control local and systemic pathologies, prevent complications, reduce mortality and morbidity rates, and reduce the economic and social burden of chronic disease. (18)

3.1. Diagnosis of oral diseases

In the eyes of dentists, the functional value of saliva has long overshadowed the diagnostic possibilities (19). Given that saliva is a reliable screening tool, saliva tests are essentially a complement to the clinical examination by the dental surgeon. Some of these tests already exist on the market but often only confirm what the physician has visually or radiologically objectified. However, caries, periodontal and cancer risks in the mouth can be more complicated to diagnose when no signs are yet visible. These saliva tests allow the detection of patients at risk and thus the implementation of early prevention strategies (32).

The foundation of modern salivary research began with oral health clinicians seeking to identify and characterize local markers of oral disease and salivary gland disorders. The discovery and validation of salivary analytes associated with human health and development encouraged expansion beyond this initial focus on individual oral pathology to include large-scale longitudinal clinical research and translational applications. (20)

In 2002, the National Institute of Dental and Craniofacial Research funded a program entitled "Saliva-Based Diagnostic Development and Validation Technologies" to support interdisciplinary research on practical salivary biomarkers for the diagnosis of systemic diseases. The program has supported studies advancing screening for pulmonary, cardiac, and infectious diseases, as well as cancer biomarker research. Much of this effort is directly or indirectly illustrated by the research presented throughout this special issue, indicating that the development and application of various salivary assays are now firmly established. (20)

The current decision to use available diagnostic methods for many conditions is based on the symptoms and clinical information described by the patient. The process of obtaining a final diagnosis can be burdensome in hospital and specifically dental office settings (21). As an accessible, noninvasive primary test for disease, salivary diagnostics can reduce the burden and use of unnecessarily invasive procedures. As methods for stabilizing whole saliva are developed, salivary diagnostics can be properly performed in dental clinics, using state-of-the-art technologies for disease detection without any preparation and through accurate, reproducible, and cost-effective testing. (21)

It is now known that oral pathologies are indicative of a disruption of the oral balance, also called homeostasis. To measure this imbalance, saliva can be analyzed as "a diagnostic window of the whole human body". In order to sensitize the dental surgeon to the identification of salivary dysfunctions, an exhaustive presentation of these salivary tests will be described, along with their field of application and their protocol.

Four different tests are used today to measure: salivary flow, saliva buffering capacity, PH, viscosity, and mutans streptococcus count. These tests can be easily performed in the dental office.

3.1.1. Quantitative and qualitative salivary evaluation

Evaluation of saliva viscosity

The viscosity of saliva is inversely proportional to the shear rate applied to it. This is a non-Newtonian characteristic common to biological fluids, difficult to reproduce in vitro, hence the difficulties in obtaining an artificial saliva that perfectly mimics these properties. The viscosity of total unstimulated saliva is not homogeneous, as the mixture of parotid, submandibular and sublingual saliva is not homogeneous. This difference in viscosity is mainly attributed to the level and type of mucins present in the different secretions (sublingual saliva is more elastic than submandibular saliva, which itself is more elastic than parotid saliva). Thus, the total stimulated saliva is much more homogeneous in terms of viscoelasticity, due to the greater importance (in volume) of the rather fluid parotid secretion. (22)

To make a visual measurement of salivary gland secretion, the practitioner asks the patient to open his or her mouth. A small amount of saliva is then grasped between the thumb and forefinger and spread under the light of the scialytic to look for capillary continuity.

The viscosity of the saliva is determined visually by asking the following question; is it watery, bubbly, or foamy and sticky?

From a prosthetic point of view, this saliva viscosity plays a role as well as the anatomical conditions (ridges, depth of the palate). The placement of a removable prosthesis would induce the reduction of salivary viscosity by a phenomenon of reduction of the salivary protein concentration.

Physico-chemical evaluation

Measurement of salivary pH
Several recent studies have shown that salivary pH can be used as a diagnostic biomarker for oral diseases and even some gastroenterological diseases. However, there is an exact correlation between salivary PH, caries and periodontal diseases. (10)

A salivary PH between 6.8 and 7.2 generally indicates a stable environment, a healthy dental and periodontal situation (10). The oral fluid becomes a saturated solution of calcium phosphates, resulting in rapid and effective remineralization of the initial changes. (32)

A salivary Ph below 6.8 generally indicates acidemia; which tends to increase the susceptibility of teeth to caries, and the oral sphere to halitosis to xerostomia and various periodontal diseases (23). However, if the environment is slightly acidified, the saliva becomes an unsaturated solution by the formation of easily soluble calcium hydrogen phosphates that promote the dissolution of the mineral phases of the dental surfaces (23). Recent research has shown that chronic acidemia may be a causative factor in a multitude of diseases affecting the entire human body (10).

A salivary Ph above 7.2 generally indicates alkalinity. Excessive alkalinity tends to promote calcium aggregation, which leads to the maturation of plaque and consequently to the development of periodontal diseases. (10)

With a multitude of biomarkers and complexities in their determination, salivary Ph can be tried for use as a rapid test in the dental office (10). The latest reports in the literature have led that the potentiometric method using PH meters is the most common laboratory technique and that this parameter should be measured immediately after saliva collection. There are also possibilities for direct diagnosis, for example in the dental chair. This is a method very simple and fast based on special test strips; the dental surgeon first asks the patient to deposit the oral fluid in a cup; then he takes a strip of Ph paper, places it in the collected sample for 10 to 15 seconds and then the data will be read from the color scale with an accuracy of 0.2 (23)

Strongly acidic saliva will be in the red zone, Ph 5.0 - 5.8. Moderately acidic saliva will be in the yellow zone, Ph 6.0-6.6. Healthy saliva will be in the green zone, Ph 6.8-7.8

This value should therefore allow an estimation of the risk factors in case of an extreme value obtained. The Saliva-Check Buffer® is one of the commercial references available from dental equipment suppliers for the estimation of salivary pH (fig.2).

Figure 2 Measurement of salivary pH with the Saliva-Check Buffer® kit (24)

Measurement of Buffer Capacity
The physico-chemical and biochemical properties of saliva as well as its complex composition give this fluid multiple functions, including: antibacterial, antiviral, antifungal properties; digestive activity and buffering capacity for plaque acids (1)

The buffer systems are responsible mainly on the maintenance of a good acid-base balance, thanks to the presence of bicarbonates, phosphates, proteins, free amino acids, ammonia and urea. (23)

In view of the modern methods of saliva testing, it is worth mentioning the possibilities of measuring the buffer capacity in the dental practice with the help of special test kits, we distinguish 3 relevant tests of measurement: (23)

- The Ericsson method: This is certainly the most accurate of the three methods mentioned; however, it is also the most time-consuming. The sample taken will first be transferred to a medical laboratory and processed. The results are then sent back to the dentist for examination. 1 ml of freshly collected saliva is transferred to 3 ml of HCl (hydrogen chloride). To avoid foaming of the solution, the dentist should add a drop of 2-octanol and mix for 20 minutes to remove the CO 2. The final pH of the solution is then measured with a pH meter (23).
- The Dentobuff Strip System method: this method is much faster; a result can be obtained within 5 minutes; however, it is neither as accurate nor as complete as the Ericsson method mentioned above. Buffer capacity is determined by the color of the test strip 5 minutes after a drop of saliva is applied to the strip (23).
- CRT® Buffer: This is definitely the fastest of the three methods with results available after only two minutes. Interestingly, it is also more accurate than the Dentobuff Strip System because it has multiple test areas. The buffer capacity is determined according to the color of the test strips two minutes after the application of saliva to the strip (Fig. 3). (23)

The CRT® Buffer is a commercially available reference that allows the rapid and efficient determination of the buffering capacity of saliva.

Figure 3 Measurement of saliva buffering capacity using the Tompon CRT® kit. (24)

3.1.2. Tests for the detection of oral diseases

Bacterial tests

The oral microbiota contains over 700 individual microbial taxa, making the oral flora one of the most complex microbial communities in the human body (1).

Indeed, researchers found that higher salivation levels of Porphyromonas gingivalis, Tannerella forsythia and Prevotella intermedia was found in people with aggressive periodontitis. This phenomenon was also noted by a recent study, which shows that the combination of the salivary amount of P. gingivalis with a host-specific pathogen response would be useful to diagnose periodontitis with high accuracy. A salivary test can detect most periodontal pathogens (MyPerioPath®, OralDNA®Labs). The patient must rinse with saline for 30 seconds and then spit into a collection tube. The samples are then sent by priority mail to the laboratory for microbiological analysis. This test has been approved for chairside use in the United States and Canada (25).

According to the Canadian Health Measures Survey (2007-2009), 96% of Canadians have at least one decayed, missing or filled tooth. Decay is the result of demineralization of the tooth surface initiated by the acid production of cariogenic bacteria. This process can eventually lead to tooth loss. Many studies have demonstrated the role of Streptococcus mutans in the initiation of dental caries, while lactobacilli have a role in the progression of its lesions. Both pathogens can be identified with a salivary test: CRT ® bacteria, Ivoclar-Vivadent Inc, Amherst, USA; for the overall assessment of caries risk in patients in dental practices. (25)

The immunochromatographic test

There is no doubt that future research to isolate genetic, microbiological and host-derived risk factors will provide a better understanding of potential biomarkers of oral disease. (25)

In the field of salivary diagnostics, recent device developments with new technologies have advanced considerably over the past decade allowing for more accurate chairside testing and improved individualized care.

In 2010, an immunochromatographic test became commercially available to determine the presence or absence of Matrix Metalloproteinase-8 (MMP-8) as a biomarker of periodontal disease with similar accuracy to conventional laboratory tests (Periosafe®) (26).

Currently, the MMP-8 lateral flow immunoassay is a recently developed mouthwash available from dental suppliers as a convenient, accurate and inexpensive test that takes only 5 minutes to perform and is used to detect, predict and monitor the progression and treatment of periodontitis (26).

The sensitivity and specificity of the PerioSafe® test have been demonstrated to be 76.5% and 96.7% for more than 2 sites and deep periodontal pockets. The test has been successfully validated independently and internationally in Africa, Europe and the United States, and in all recent studies and research (25) It also qualifies the biomarker MMP-8 which could identify disease activity, predict progression and monitor response to treatment (fig.4 and 5). (16,25)

A line on the test device indicates that the test has successfully analyzed the saliva drop and the result is negative. (16)

Two lines are observed on the device indicating a high risk of periodontitis and that the result is positive. (16)

Figure 4 Saliva test kit: Periosafe® used to detect the presence of aMMP-8 in patients with periodontitis. (16)

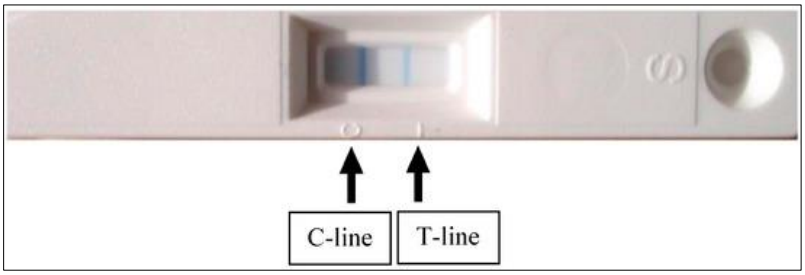


Figure 5 Two lines (control line C; and test line T). (16)

Autres tests

Other tests exclusively performed in the laboratory exist and have only minor interest in daily practice. Such as, the analysis of protein composition by gel electrophoresis which allows the separation and identification of proteins with more precision in order to resolve the complex composition of saliva, contribute to a mapping of the protein elements present and the development of a wider range of applications for proteomic tests. (27)

Real-time quantitative qPCR (quantitative Polymerase Chain Reaction) can also be used and constitutes the gold standard for genomic analysis of saliva. It is perfectly suited for the validation of transcriptomic biomarkers after microarray profiling, and it is not limited by the length of the RNA, even for fragmented RNAs. However, their low quantities in saliva considerably hamper the performance of the test (27). To overcome this problem, DNA Labs, an American company specializing in saliva testing, suggests that the patient sends a sample of his or her saliva. The patient can choose to have his or her saliva tested, even for paternity tests, which is more a matter of folklore than a necessity in the daily life of a health care professional. (25).

3.1.3. Detection of viral diseases

COVID 19

"Combating infectious diseases by learning from Covid 19: relaunching the pandemic plan and regaining momentum in the field of testing. (28) "

Since the outbreak of pneumonia caused by the novel coronavirus (SARS-CoV-2) (COVID-19) occurred in December 2019, it has spread rapidly around the world and caused a significant threat to global human health (5). Clinical

symptoms were similar to viral pneumonia, including fever, dizziness, and cough. After sequencing analysis of samples obtained from the respiratory tract, the pathogen was identified as a novel coronavirus (coronavirus 2, SARS-CoV-2, COVID-19), causing severe acute respiratory syndrome due to phylogenetic similarity to SARS-CoV (29). Case identification by testing has been a key pillar of the global response, with an emphasis on molecular reverse transcriptase-PCR testing, which remains the gold standard for diagnosis, surveillance, and monitoring of the epidemiological dynamics of the epidemic in all countries. (30,31)

The health authorities, including the High Authority of Health has authorized salivary tests, it is still a test that is done in the laboratory, but the sample is taken at the point of care, it is this cotton swab woven swabs that goes to the bottom of the nose, which is painful and unpleasant, will be replaced by a sampling of saliva much more superficial and non-invasive, it is the same technique of PCR, this amplification of the viral genome to go and look for the virus and immunological responses induced by the infection (28). With this objective in mind, the FDA has granted emergency use authorization (EUA) to several in vitro diagnostic tests, namely molecular and antigenic tests. (32)

Early investments in new diagnostic technologies with rapid and decentralized tests have been critical to minimizing the negative health and socioeconomic impacts of SARS-CoV-2. In April 2020, the U.S. National Institute of Health (NIH) launched the Rapid Acceleration of Diagnostics (RADx) initiative to accelerate the development, commercialization, and implementation of COVID-19 testing technologies. (32)

The goal of this initiative was to develop innovative tests that are rapid, accurate, specific, and easily accessible in the home and dental office, particularly for vulnerable populations most affected by COVID-1(32) While Real-Time Polymerase Chain Reaction (RT-PCR) testing remains the gold standard, more rapid or affordable molecular and antigenic testing options have been developed. (32)

Molecular salivary tests for SARS-CoV-2

In their meta-analysis published in The Lancet Infectious Diseases, Nicole Ngai Yung Tsang and colleagues compared the diagnostic performance of different clinical specimens, including nasopharyngeal, nasal, throat, oropharyngeal and saliva swabs. Using nasopharyngeal swabs as a reference, they found that these gave the highest sensitivity (97%) among alternative sampling approaches, while moderate sensitivities were achieved by saliva (85%) and nasal swabs (86%) and much lower sensitivity of pharyngeal swabs (68%).(31) The authors thus concluded that nasopharyngeal swabs salivary swabs are a promising alternative to nasopharyngeal swabs for the diagnosis of SARS-CoV-2, given the ease of collection and convenience of repeat testing as well as rapid, accurate and highly reliable results. (33)

Future in vitro salivary testing in dental offices may shed light on several key aspects of COVID-19 infections, including temporal patterns of viral loads in mildly symptomatic or asymptomatic patients and the longevity of protective immunity provided by antibodies to SARS-CoV-2 (32).

Molecular bioassays are a rapidly developing field, based on the latest research in molecular biology, and have been used for the first time on a global pandemic scale (28). Detection of viral nucleic acids from genetic material is the gold standard in diagnostic virology (34). Analytical performance data, i.e. sensitivity, specificity, lower limits of detection, for all commercially available molecular tests are provided by FIND: a global diagnostic alliance, which aims to ensure equitable access to reliable screening in hospital settings, specifically dental offices, worldwide (34). (34)

In April 2020, Infinity BiologiX (IBX) received the first emergency use approval from the FDA for its salivary tests for SARS-CoV-2. Sample collection is performed by the dentist, using the IBX collection kit (Fig. 17) or the SDNA-1000 device, which is a funnel attached to a 6 ml plastic tube. The saliva samples are then shipped to the laboratory for storage, purification of genetic material, PCR amplification of the extracted RNA and quality controls. IBX acts as a service provider for organizations with high-volume testing needs where further processing of saliva samples is performed in the laboratories (Fig. 6) (32). However, SARS-CoV-2 RNA in salivary samples is only detectable during the acute phase of infection. Positive test results suggest that SARS-CoV-2 RNA is present, but cannot exclude infections with bacteria or other viruses. Negative results from saliva samples may not exclude SARS-CoV-2 infection and should be confirmed with other samples and test methods. (32)

Figure 6 SARS-CoV-2 molecular test, Infinity BiologiX (32)

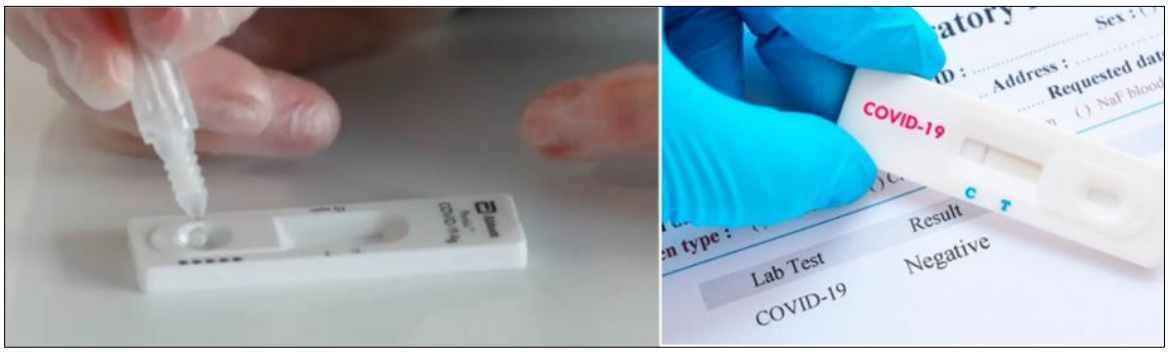
Rapid antigen tests for SARS-CoV-2

In 2020 the World Health Organization (WHO) recommended the use of rapid lateral flow antigen detection and diagnostic tests on nasal, pharyngeal and salivary swabs (35). This is a qualitative method for detecting certain proteins on the surface of the infectious agent that are capable of eliciting an immune response and that signal the presence of the virus (28,36). They have been developed as point-of-care tests administered by healthcare professionals with easy conduct and reading of results.

To confirm, isolate and manage each case in a timely manner (36). Antigen testing has received emergency use approval by the FDA (32). It involves characterization of the etiologic agent, to understand the epidemiology, detect subtle infectivity patterns, and map the transmission dynamics of new variants. (32,36)

Although direct antigenic tests are increasingly used, their diagnostic accuracy is lower than that of RT-PCR (37). There are some concerns about their specificity, as they may cross-react with other coronaviruses, but the main problem is their sensitivity (38). The sensitivity of these panels ranges from 34% to 88%, based on the results of a Cochrane systematic review (37,39). Therefore, despite the greater accuracy of positive results, negative results should be treated with more caution. Their diagnostic accuracy is increased in symptomatic patients, when the viral load in saliva is higher, allowing for early diagnosis, triage and treatment (40). Optimal timing of testing may vary within 5, 7, and 12 days of symptom onset (41). Therefore, the advantages of point-of-care testing, namely the rapidity of results (5-30 min) and low cost (Dh100-150) compared with RT-PCR testing, may be limited to some extent by the low sensitivity it offers, particularly in asymptomatic or presymptomatic individuals (42). Overall, antigenic tests appear to be particularly well suited for screening, and dysfunctional tracing by the dental physician, in the management of patients for specific signs of Covid 19 infection (34). Different antigen test kits are produced by manufacturers of diagnostic reagents in different countries, namely the Gigalab® for the detection of coronavirus on salivary samples (fig.7 and 8). (43)

Figure 7 Gigalab® saliva test kit for detection of SARS-CoV-2 (43)



a) A small amount of saliva is collected and rubbed onto the commercial reagent; b) C-line control: result is negative; and T-line test: result is positive.

Figure 8 Rapid antigen tests for SARS-CoV-2 (44,45)

This pandemic illustrates that the world needs to be much better prepared to rapidly detect, define and defeat future pandemics. To do this, we need to bring together information that is currently scattered, and create the large databases from past episodes and new tools that will allow us to quickly reach a preliminary understanding of the dynamics of immunity upon the arrival of a new infectious agent; thus creating prognostic algorithms based on artificial intelligence without having to resort to invasive, expensive and unpleasant sampling. (28)

HIV

Diagnostic tests for viral infections currently rely on salivary biomarkers, such as DNA, viral RNA, antigens and antibodies (3). Oral samples can provide an ideal matrix for diagnosing many viral infections including HIV (46). Results of several studies have shown that the sensitivity and specificity of these oral tests are comparable to those of plasma and urine (47)

When oral symptoms suggest it, or following the patient's request, the dentist can conveniently initiate a rapid salivary antibody test for the screening and diagnosis of HIV (46). The US Food and Drug Administration (FDA) approved in 2004 the OraQuick® HIV 1/2 test which involves the use of the commercial slide in dental offices (47). This test is capable of detecting the decline in salivary HIV IgA levels when patients are infected. It has been suggested that the detection of HIV IgA antibodies in saliva may therefore be a prognostic indicator of HIV infection. (46)

The OraQuick® HIV 1/2 Rapid Advance consists of a fairly rigid swab attached to a lateral flow immunochromatographic (LFT) test strip (48). The physician swabs the area under the lips and around the top of the gum line for a few seconds to collect an adequate sample. The sampling device is then immersed in a buffer/reagent solution; after 20 min, the test results (qualitative) are read. If only one line is observed, the sample is negative. If two lines appear, the result is considered "preliminary positive," and the patient is referred immediately to a clinic for

confirmatory testing of the virus (Fig. 9) (46,48). The performance of OraQuick Advance® is equivalent to or better than many FDA-approved ELISAs for the HIV virus and has become a diagnostic standard at the point of care and in hospital settings. (48)

Figure 9 OraQuick Advance® Saliva Test for HIV (48)

Human papillomaviruses

The Human Papillomavirus (HPV) is a DNA virus (47) that infects the epithelial cells of the skin and mucous membranes (46). Researchers have identified more than 100 different types capable of infecting the genital tract and oral cavity as well as causing warts primarily on the hands and feet (46).

Salivary diagnostic tests are available to detect HPV, which primarily involve the use of point-of-care PCR. Kits containing a salivary collector are placed in transport media and sent to a central laboratory for analysis (46). The salivary test, OraRisk human papillomavirus, is the only commercially available diagnostic test that identifies several types of this virus, with an emphasis on human papillomavirus-16 and human papillomavirus-18, the types most commonly linked to oral cavity cancers (fig.10) (47). The laboratory researchers used a variety of primers to detect as many types as possible. Early diagnosis is therefore crucial to improve survival in at-risk patients, and therefore gene sequencing and profiling of specific proteins in saliva may be an attractive avenue for future HPV diagnosis and monitoring. (35)

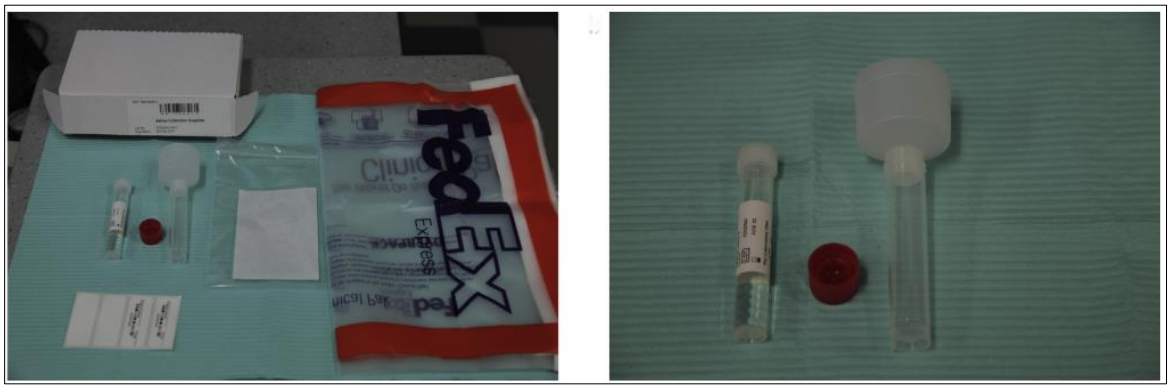


Figure 10 OraRisk human papillomavirus saliva test kit used to detect the presence of HPV-16. (25)

3.1.4. Detection of endocrine, nutritional and metabolic diseases

Diabetes mellitus

Diabetes is a multifactorial metabolic disease characterized by chronic hyperglycemia and disorders of carbohydrate, lipid and protein metabolism. It is caused by a defect in insulin secretion (type I), insulin action (type II), or both (55). Hyperglycemia is an effect of uncontrolled diabetes and over time can lead to severe damage to many organs in the body, primarily blood vessels and nerves (16). Therefore, an oral test to monitor blood glucose would be highly desirable (1). In this regard, saliva has been explored as a substrate to measure glucose levels in diabetes. Preliminary studies have shown that it could serve as a potentially noninvasive adjunct to monitor glycemic control in diabetic patients. (50)

A recent report by Rao et al. demonstrated a unique proteomic signature in saliva obtained from type 2 diabetic patients (1). The authors found that 52 proteins were differentially expressed and higher levels of certain diabetes-related inflammatory biomarkers were observed in the saliva of patients compared with controls. Other researchers reported that of a total of 487 proteins analyzed in oral fluid, 65 had higher levels in type 2 diabetic subjects compared with healthy individuals (25,51). This high protein content makes saliva more attractive compared with other body fluids for the detection of biomarkers of hyperglycemia in biomedical monitoring within dental offices. (52)

Due to the ease of its collection, and the advancement of nanotechnology, the field of salivary detection has undergone considerable progress, aimed primarily at the incorporation of in vitro diagnostic sensors on strips or platforms of portable devices. (50)

The Mouthguard is a miniaturized oral biosensing device in the form of a detachable wireless transmitter of "cavitas sensors" to measure salivary glucose levels, which is perfectly integrated into a mouthpiece fabricated to fit the wearer's teeth (fig.11) (52,53). This configuration allows for telemetric measurement, and continuous glucose monitoring from an alarm that is well incorporated into the saliva sensor and automatically alerts the patient when the glucose level is above or below a predefined threshold (50).

As demonstrated salivary glucose has been shown to have a positive correlation with blood glucose levels. This correlation reflects the diffusion and active transport of blood components to the salivary gland. Soni et al. demonstrated that the correlation between blood and saliva glucose concentrations was R = 0.64 in healthy subjects, while diabetic subjects showed a much closer relationship with R = 0.95. However, further large population studies are needed before the use of salivary glucose for screening or monitoring of diabetes in integration with a miniaturized portable platform can be considered (Fig. 11) (52).

Considerable effort has been invested in the development of biosensors capable of detecting glucose in saliva rather than in blood. Demonstration of reliability and thus validation of all methods for diabetes diagnosis is ongoing in many research teams, therefore measuring qualitative and quantitative alterations in saliva could be a promising, less invasive and less expensive way to monitor affected patients. (54)

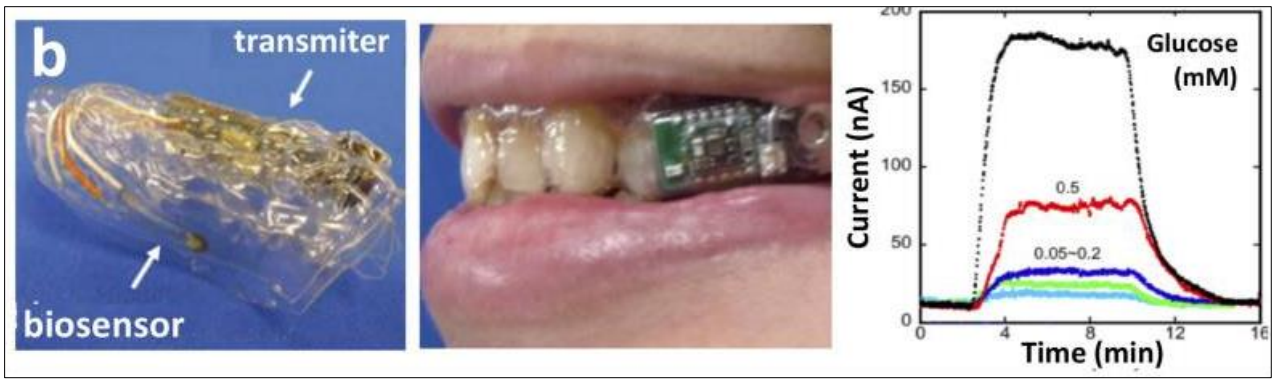


Figure 11 Saliva glucose sensor "mouth guard". (52)

Obesity

Childhood obesity is a worrying scourge affecting our society. Salivary biomarkers have recently been explored as potentially useful screening tools in patients diagnosed with metabolic disorders, namely obesity or diabetes (55-57).

The urgent need to control the global obesity epidemic draws attention to at-risk pediatric populations for the application of preventive strategies, which include dietary recommendations and physical activity suggestions. Moderate exercise in children has been found to decrease the incidence of infections (51). Salivary IgA, an immune biomarker also known as the "first line of defense" against pathogens, was upregulated in children after moderate exercise and downregulated after excessive exercise (58). According to the reported study, after examination of 132 children, Body Mass Index (BMI) was found to be an independent predictor of salivary IgA secretion rate (58). In another study of 170 South African children, reactive protein-c (CRP) concentrations showed that obese children had significantly higher salivary CRP values than the normal weight control group [63]; Cook et al. examined 699 children and found that obesity is a major determinant of CRP levels, which were also significantly correlated with several cardiovascular risk factors. Similar findings were reported by Goodson et al in 2014 (59) who studied the risk of metabolic disease in 744 11-year-old children. The alterations in salivary CRP, salivary insulin, leptin, and adiponectin levels that were observed in this large cohort suggest that salivary biomarkers could be used for identification of vulnerable subjects (59). The above reported alterations in salivary biomarkers in children and adolescents may suggest the initiation of metabolic changes associated with obesity and diabetes. However, because of the complexity of the interactions of biological pathways, further research and longitudinal studies are needed before any of these markers can achieve precise diagnostic value (51,56).

3.1.5. Detection of autoimmune diseases

Gougerot-Sjögren syndrome

Gougerot-Sjögren syndrome (GSS) is a chronic systemic autoimmune disease characterized by keratoconjunctivitis sicca and xerostomia. With further development of GSS, salivary flow decreases and salivary constituents change (3).

Diagnosis and detection is usually based on a series of clinical and histopathologic signs and symptoms that are often difficult to interpret, and different classifications have been published. This has prompted research into the diagnostic and prognostic value of salivary biomarkers in this condition to facilitate early treatment and reduce associated complications. (60)

Recent proteomic studies have reported increased salivary Interleukins (IL) such as IL-2 and IL-6, reduced stimulated and unstimulated salivary flow, and increased IgA, IgG, IgM, Na, lactoferrin, albumin, microglobulin, cystatin S and C, lipids, prostaglandin E2, and thromboxane B2 in patients with primary and secondary Sjögren's syndrome. (61)

In the very near future, biomarker panels will likely gain the specificity necessary for saliva to be useful as a true diagnostic fluid within dental offices. However, this will only be achieved once the right combination of markers is validated in longitudinal studies and their reliability is confirmed against daily cyclical variations and potential confounders (8). In this line, the combination of three mRNA biomarkers (myeloid cell nuclear differentiation antigen, guanylate-binding proteins 2, and low-affinity receptor IIIb for IgG Fc fragment) and the three protein biomarkers (cathepsin D, A-enolase, and β2-microglobulin), is a step closer to validating a diagnosis of GSS from salivary tests. The synthesis of proteome and transcriptome data could lead to the development of a low-cost, simple clinical tool for the diagnosis of primary and secondary SMS in dental offices. (61,62)

Cystic fibrosis

Cystic fibrosis (CF), also known as cystic fibrosis of the pancreas, is one of the most common inherited diseases in Caucasians. It usually leads to premature death from respiratory complications. Mutations in the cystic fibrosis transmembrane conductance regulatory protein are involved in the chronic inflammation process occurring in the lungs of affected patients. (25)

It is suggested that saliva can potentially be used to diagnose cystic fibrosis as an alternative to the sweat test (63). As it is a valuable source of clinically relevant information, which act as biomarkers of disease and systemic conditions of the individual (64).

The saliva of cystic fibrosis patients represents increased levels of calcium and phosphate, which may explain a higher incidence of tartar observed in these individuals. These patients also have higher salivary levels of chloride, potassium and sodium ions with lower salivary volume and pH than healthy individuals. In addition, whole saliva samples from young cystic fibrosis patients have higher levels of protein, antioxidants and uric acid compared to controls. Javaid et al. reported in their study that all these salivary changes are related to the chronic activity of oxidative and inflammatory processes in the oral cavity of these patients and represent biomarkers that: can give more clues about the etiology and follow-up of cystic fibrosis. (25)

Detection of proteins, ions and bacteria in salivary samples can help prevent or delay chronic lung infection in VD. InPlex from Hologic ® (Bedford MA), is a molecular salivary test used in dental offices to simultaneously detect 23 mutations in the cystic fibrosis system and the fibrosis transmembrane receptor (CFTR) gene (48), it is an easy, It is an easy, rapid and feasible test that appears as a potential alternative to identify patients at risk and in need of specific management, which in turn could open a window of opportunity to eradicate the etiological factors in the oral compartment before they are aspirated into the lungs. (65)

3.1.6. Detection of malignant tumors

Cancer of the oral cavity

In oncology, early diagnosis and treatment are the key to a good prognosis in almost all types of cancer. Saliva has been used in several studies as an early diagnostic tool for oral squamous cell carcinoma (OSCC) based on salivary analytes (proteins, mRNA and DNA). Oral cavity cancer is the sixth most common cancer type worldwide, of which 90% is represented by BEC. The average 5-year survival rate is approximately 60%, and the high mortality rate is generally associated with late diagnosis (3).

In disease diagnosis and health surveillance, the use of salivary tests has proven to be a promising and adequate alternative to blood tests for the detection of oral cancers, which are in direct contact with the oral biofluid. (66)

To date, several biomarkers have been reported in association with BEC, including IL-8, B-type endothelin receptor hypermethylation, and microRNAs (such as miR-200a, miR-125a, and miR-31). Other previous salivary transcriptomic studies found seven RNAs associated with oral squamous cell carcinoma (S100 calcium-binding protein P, dual specificity phosphatase 1, interleukin-8, interleukin-1beta, H3 histone family A3, ornithine decarboxylase antizyme 1, and spermine N1-acetyltransferase) with a predictive accuracy of 81% as biomarkers for OSCC. Other research studies have proven the importance of three tumor markers (Cyfra 21-1, tissue polypeptide antigen (TPA) and cancer antigen CA125) that have been shown to have high levels in the saliva of patients diagnosed with BEC. (3)

The onset and development of malignancy is related to somatic mutations in tumor-specific DNA, which can be found in saliva, plasma, or other body fluids. These somatic mutations can be used as biomarkers to diagnose oral and other tumors. In saliva, tumor-specific DNA was positive in 100% of oral cancer patients. However, only 47% to 70% of patients with tumors in other parts of the body were positive. Based on these results, Park et al. found that salivary tumor-specific DNA has the potential to be used to diagnose cancers of the oral cavity. (62)

In addition, salivary diagnostic tests can also be used to identify certain tumor proteins that constitute biorecognition molecules of oral squamous cell carcinomas. In this sense, several studies have reported that the increase of the cancer antigen CA15-3 and antibodies for the tumor protein markers c-erbB2, CA-125 and P53 in the oral fluid can be considered as salivary biomarkers of oral and extraoral cancers; thus, representing the bio-indicating power of these analytes in the screening, diagnosis and management of patients with oral cavity cancers in dental practices. (3)

With the development of high-throughput sequencing technology, The Collaborative Oral Fluid Diagnostic Research Center, in partnership with engineers from the University of California, Los Angeles (UCLA) have developed an electrochemical detection platform based on micro- and nano-electrical-mechanical system biosensors, capable of real-time, ultrasensitive multiplex detection of salivary proteins and RNA biomarkers. This proposed product has been labeled Oral Fluid NanoSensor Test (OFNASET®) (19). OFNASET® is an integrated, automated, easy-to-use point-of-care system that allows simultaneous and accurate detection of multiple salivary proteins and nucleic acids specific to each cancer type. Moreover, this system is portable and could be used not only in dental offices, but also in any other health care station to perform instant point-of-care diagnosis (fig.12). [16]

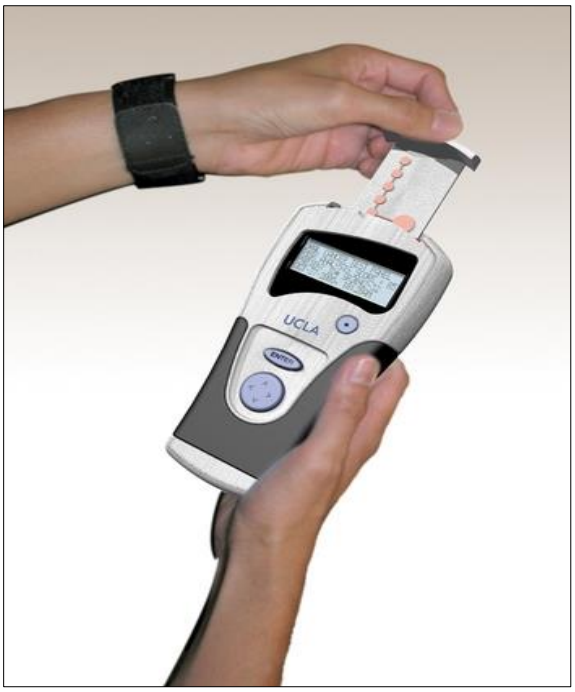


Figure 12 UCLA OFNASET® salivary sensor for oral cancer detection (19)

Breast cancer

Breast cancer is one of the most common cancers in women (62). Traditional screening mammography is considered the gold standard for diagnosis of breast cancer, but the sensitivity of this test varies with the type of mammogram. Other biomarkers of this cancer are being investigated: ATP6AP1 is an ATPase that is expressed in normal tissues such as the brain marrow, blood, nerves and skin, and it is also correlated with several tumors such as head and neck carcinomas, lung tumors, adrenal tumors and various other cancers. Nevertheless, its prevalence in breast cancer is the highest among all these cancers, which may contribute to its early detection. (67)

In their study in 2016 Zhang et al. found eight mRNA biomarkers and one protein biomarker that could be used to detect breast cancer with a sensitivity of 83% and specificity of 97%. In another study, levels of vascular endothelial growth factor, epidermal growth factor (EGF) and carcinoembryonic antigen in saliva were found to be significantly increased in breast cancer patients (62). CA15-3 and c-erB-2 levels were also increased in saliva, which has positive correlations with the serum of breast cancer patients (68). Based on these studies, potential salivary biomarkers can be applied to early diagnosis of breast cancer (62). However, to date, the evidence remains too limited to confirm the use of salivary biomarkers as diagnostic tools for this cancer. (62)

Lung cancer

Lung cancer is the most common cause of death in men and women worldwide (21). Because most of the symptoms seen for this type of cancer are nonspecific, diagnosis is usually made late or at an advanced stage. Despite advances in the management of this type of cancer, it remains a significant global health burden with survival rates that have not significantly improved in recent decades. The reduction in mortality with low-dose helical CT (LDCT) screening of high-risk patients is challenged by the high false-positive rate and potential morbidity associated with follow-up diagnostic evaluation in patients at high risk for iatrogenic complications. The diagnostic dilemma of this indeterminate nodule has created the need to search for reliable biomarkers and credible salivary tests that are easy to use, noninvasive, cost-effective, and feasible within the data practices. (69)

Using two-dimensional difference gel electrophoresis and mass spectrometry, Hua and others performed a proteomic analysis of saliva from lung cancer patients. Sixteen candidate biomarkers were discovered and further verified in the saliva sample, and three candidate markers achieved a sensitivity of 88.5% and a specificity of 92.3%. (70)

The salivary transcriptome was also analyzed in a cohort of 42 lung cancer patients and 74 healthy controls by microarray. Seven mRNA transcripts [BRAF (murine v-raf sarcoma viral oncogene homolog B1), CCNI (cyclin I), EGFR, FGF19 (fibroblast growth factor 19), FRS2 (fibroblast growth factor receptor substrate 2), GREB1 (growth regulation

by estrogen in breast cancer 1) and LZTS1 (leucine zipper, putative tumor suppressor 1)] expressed in saliva were identified and pre-validated. The logistic regression model with the combination of five mRNA biomarkers (CCNI, EGFR, FGF19, FRS2, and GREB1) was able to differentiate lung cancer patients from control subjects with a sensitivity of 93.75% and specificity of 82.81% (71). These results highlighted salivary biomarkers that require validation in prospective multicenter studies for definitive use as a noninvasive lung cancer detection tool.

The team led by Wei et al. developed a novel core technology, electric field-induced release and measurement (EFIRM), which can detect epidermal growth factor receptor (EGFR) mutations directly in body fluids, including saliva (69). It is an electrochemical analysis approach based on attached nucleic acid probes capturing mutated sequences after the application of electric fields to facilitate the hybridization process. Because of the speed and simplicity of the method, EFIRM could be a potentially interesting tool for monitoring oncogenic mutation in patients. A blinded test was performed on saliva samples from 40 patients with non-small cell lung carcinoma (NSCLC). EFIRM detected exon 19 deletion and L858R mutations in saliva and plasma samples from lung cancer patients, and showed slightly higher overexpression of the L858R mutation in saliva than in plasma, reinforcing the value of saliva testing for more accurate and specific early detection of lung cancer (69).

Pancreatic cancers

Pancreatic cancer is the fourth leading cause of death in men and women of all ages worldwide with a 5-year survival rate of 3-5% compared to other cancers. It has been estimated that this disease causes over 40,000 deaths per year in the United States. Nearly 100% of patients with pancreatic cancer develop metastases due to late presentation, lack of effective treatment protocols, and early detection tools.(69)

Zhang et al. profiled the salivary transcriptomes of 42 patients with pancreatic cancer, and 42 healthy control individuals using the Affymetrix HG Plus 2.0 array (72). Their results showed that the combination of 4 salivary mRNA biomarkers (KRAS, MBD3L2, ACRV1 and DPM1) could differentiate cancer patients from healthy subjects with a high sensitivity of 90.0% and specificity of 95.0% (69).

According to the team led by Kaczor-Urbanowicz, the discriminatory power of salivary miRNAs could differentiate patients with non-operable pancreatic tumors from those with precancerous lesions, inflammatory diseases or control subjects (73). He also showed the importance of miRNAs in salivary diagnostics. The best candidates are 5 miRNAs (miR-17, miR-21, miR-181a, miR-181b, and miR196a) that were differentially expressed in salivary samples from diseased patients. Wang and his team report that among 94 salivary miRNAs examined in patients with pancreatic cancer, pancreatitis, intraductal papillary mucosal neoplasia, and healthy controls, the transcriptomes miR-21, miR-23a, miR-23b, and miR-29c are significantly present in the saliva of pancreatic cancer patients compared with controls, showing perfect specificity (100%) but with low sensitivity between 57% and 85.7% (69). Microarrays were used to assess salivary miRNA from patients with resectable pancreatic cancer. The combined miR 3679-5p and miR-940 model distinguished pancreatic cancer resectable, with sensitivities of 72.5%, 62.5%, 70.0% and specificities of 70%, 80% and 70% respectively. (69)

In their study Farrell et al. observed significant variation in salivary microbiota between 10 pancreatic cancers and 10 control subjects using Human Oral Microbe Identification Microarray (HOMIM), later validated using quantitative PCR in an independent cohort. The combination of Neisseria elongata and Streptococcus mitis provided a sensitivity of 96.4% and a specificity of 82.1% to distinguish pancreatic cancer patients from healthy subjects. These reports open a new avenue for the salivary microbiota as a credible source of information to discover non-invasive biomarkers that add to those already demonstrated and reinforce the value of salivary testing for more accurate and specific early detection of pancreatic cancer (69).

3.1.7. Mental and behavioral disorders

Stress

Stress is the non-specific response to any demand related to the body's adaptations, through the activation of the sympathetic and para-sympathetic nervous system. In psychology, two types of stress are often distinguished: positive and negative. Positive stress (eustress) helps to improve the individual's performance and motivation, whereas, distress (negative stress) is considered as an excessive amount of stress, mainly related to the tension and emotional pressure of the individual which can lead to serious health risks, and dramatic consequences in private and professional life. (74)

Observation of the patient and medical interviews conducted with him/her and his/her immediate family members are currently the main methods used to diagnose stress. Moreover, the multitude of causes and the different symptoms that

overlap with other mental disorders often make it difficult for psychiatrists to make an appropriate diagnosis and apply effective treatment (75). Therefore, salivary components may be useful in this setting, via promising and potentially indispensable biomarker tests for the prevention, screening and monitoring of patients with acute and chronic stress (75).

The most commonly considered biomarker for the determination of stress in humans is cortisol [79], which is a hormone produced by the adrenal cortex primarily in the second half of the night, meaning the highest levels between 7:00 and 8:00 am (75). When a person is exposed to mental or physical stress, the adrenal glands produce increased amounts of cortisol which activates the body's metabolism, provides energy through the release of glucose and alters the conditions of mental reactions (75). In their meta-analysis of the relationship between stress and hypothalamic-pituitary-adrenal (HPA) axis activity, Miller et al. confirmed the importance of salivary cortisol as a biomarker of acute stress, showing the close relationship between acute stress and the concentration of this hormone in saliva (76).

Other salivary biomarkers that may be useful for acute stress screening include Alpha-Amylase (SAA). It is considered the main digestive enzyme of the oral cavity, and in addition to hydrolyzing starch and glycogen, also has an immunological function, protecting the oral cavity from microorganisms (75). The usefulness of alpha-amylase as a salivary biomarker of acute stress and anxiety was confirmed by Jafari et al. in 2018 highlighting its reliability and objectivity in measuring anxiety associated with dental care (77). In turn, Van Veen et al. suggested that SAA could be a potential marker of salivary stress in individuals sensitive to negative social evaluation. (75)

A recent study by Vivek Shetty and his team presented validation of the development of a portable point-of-care biosensor system for rapid measurement salivary α-amylase levels. A simple disposable colorimetric test strip allows for simplified sample collection and preparation, combined with a portable reader with digital display (Fig. 13).

The test strip incorporates a pad at the end and a reagent paper placed under the individual's tongue. After 10 seconds, the strip is inserted into the reader. The Microprocessor Unit (MPU) of the biosensor calculates the SAA level and displays it as a numerical value with a time stamp. From collection to reading, the duration of the entire test is approximately 30 seconds. (78)

Figure 13 Portable sAA biosensor comprising a handheld reader and a disposable collection strip. (78)

At this time, it may be too early to introduce most of these biomarkers into the routine daily diagnostic applications of the dental physician, but advances in the standardization of salivary biomarkers should allow for their widespread use in the future, including safe, reliable, and noninvasive estimation of acute and chronic stress levels in patients. (79)

Depression

Over the past decade, the rate of affective disorders has increased worldwide. Research reports suggest that depression is the second most common disease after heart failure and may even take the lead by 2030. Depressive disorders are very often preceded by stress and accompanied by anxiety; the coexistence of anxiety and depression is found in nearly 75% of children and adolescents. However, if anxiety and depressive disorders do not occur at the same time, they generally follow one another. According to the team led by Chojnowska et al. the probability of Anxiety disorders after a depressive episode is 47-58%, and 56% of patients with anxiety disorders develop depression. (75)

There is a growing interest in the biological underpinnings of depression, which are reflected in altered levels of salivary markers. Among other things, increased inflammation has been reported in major depressive disorder, as evidenced by

increased levels of inflammatory markers, serum cortisol, interleukin-6, tumor necrosis factor-α, soluble interleukin-2 receptor, and C-reactive protein. (80)

Elevation of serum cortisol is found in many patients with major depressive disorder and may be due to chronic dysfunction in the feedback control of the pituitary-pituitary-adrenal axis. Salivary cortisol is a valid indicator of serum cortisol. A higher level of cortisol in saliva confirms depressive symptomatology over time in unipolar major depressive disorder. Higher cortisol secretion is associated with a more chronic pattern of depression. (81)

Recently, measures of testosterone in saliva have been widely used in the assessment of the degree of aggression, depression, violence and antisocial behavior in psychiatry. (61)

In August 2013, Oasis Diagnostics® received a research and innovation grant to complete the development of a human salivary cortisol test with its rapid point-of-care platform under the name VerOFy® (48).

VerOFy® is a commercial device that combines rapid, standardized oral fluid collection with high-quality immunochromatographic test strips, placed under the tongue for a period of time until a sample volume adequacy indicator (built into the device) changes appearance (pale yellow-green to dark blue). The device is removed from the mouth and the results are available immediately in the hospital setting after 10 to 15 minutes (Fig. 13). (48,82)

VerOFy® cortisol is a rapid salivary test configured for instrumental reading, through, the LIAM™ (Light Module for Image Analysis) portable scanning module. The LIAM™ is designed to archive a limited number of results and can also offer the ability to transfer files directly to a smartphone or Bluetooth enabled device. LIAM™ is battery powered, lightweight, portable, and capable of operating in hard-to-reach locations (fig.13). In addition to salivary cortisol, the VerOFy® platform can be configured to assess multiple biomarkers such as testosterone and other hormones simultaneously in a quantitative manner. (82)

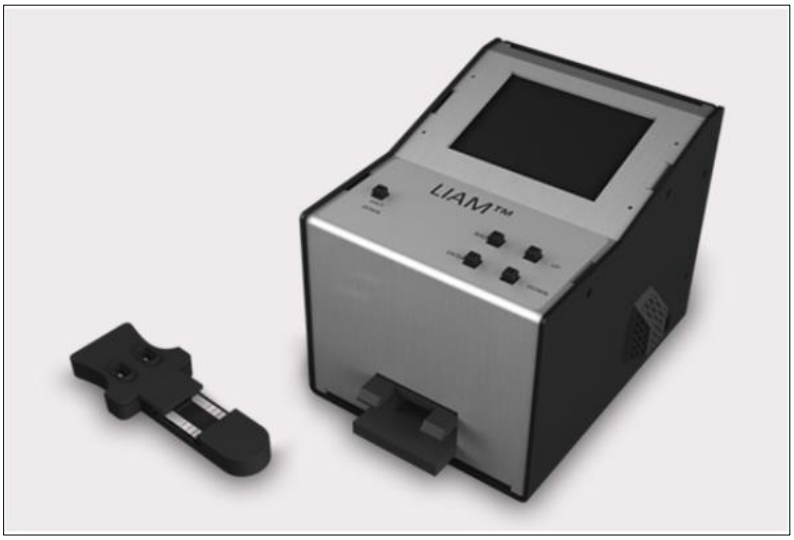


Figure 14 VerOFy® and LIAM™ rapid saliva test for measuring serum cortisol (82)

Migraine

Chronic migraine is a disabling neurological condition that affects 2% of the general population. It imposes an enormous burden on patients due to frequent headaches; hypersensitivity to visual, auditory and olfactory stimuli; nausea; and vomiting. It also affects society through direct and indirect medical costs. Diagnosis requires a carefully conducted patient interview and neurological examination, sometimes combined with additional diagnostic tests to differentiate chronic migraine from secondary headaches. Among the complementary tests we distinguish the non-invasive salivary tests performed by the dentist in the dental office for the early detection of specific biomarkers related to this pathology. (83)

Jang and his team observed increased levels of nerve growth factor (NGF) and sensory neuropeptides (including substance P and calcitonin gene-related peptide [PRGC]) in the saliva of the individuals studied. These biomarkers are strongly correlated with pain severity in patients diagnosed with chronic migraine. The results of the saliva analyses

could, upon further investigation, serve as an index of disease status and therapeutic outcome in patients with chronic migraine (84).

These biomarker-based findings do show that they may have a future role in helping to target specific treatment for migraine to guide the dentist in managing patients within the dental office. However, the researchers indicate that they are not yet ready for affordable, accurate salivary testing that can modify current procedures.

4. Technological advances in salivary diagnostics: looking ahead

The hunt for disease-related biomarkers in saliva is currently being pursued with great energy (85). The combination of different omics fields represents the future of salivary diagnostics. These fields have already transformed various approaches, such as risk assessment, screening and therapeutic management, for a variety of biomedical applications (7). In the near future, salivary tests will be further refined to assess various diagnostic biomarkers at early stages minimizing progression to advanced stages. (7)

With the increased emphasis on prevention and early detection of a variety of diseases, the development of small wireless devices has had a significant impact on health services. The next decade will see breakthroughs in accuracy, efficiency, and monitoring at the bedside rather than in the hospital setting. (86)

Recently, due to the combined integration of miniaturized diagnostic technologies, salivary biosensors, lab-on-a-chip systems, individual genetics, monitoring parameters, smartphones, and microfluidic devices, a large number of medically useful analytes in saliva are being progressively unveiled and some of them represent biomarkers for various diseases including cancer, autoimmune diseases, viral diseases, bacterial diseases, metabolic diseases, and HIV (86,87). These futuristic technologies have the ultimate goal of expanding salivary diagnostics and improving the primary care system in dental offices (88). In addition, they allow clinicians to be more accurate, more consistent, capture clinical data quickly, ensure patient satisfaction, and streamline workflow. The impact of salivary diagnostics on the healthcare system is enormous, as it is non-invasive, convenient, and well-accredited, while the introduction of bioinformatics will make standards and performance increasingly high and improved. (86)

In the last decade, the advancement of nanotechnology and the nanochip gives hope that in the very near future patients at risk will be monitored in real time and above all in a less invasive, less expensive and very accurate way. This allows us to dream of the creation of a biological nano-sensor, incorporated into a tooth in contact with saliva and in the service of health. (89)

In 2012 a major contribution was proposed by Mannoor et al. to realize a "connected tooth" (89). These pioneers invented a dental tattoo for continuous wireless monitoring of bacteria by biofunctionalizing antimicrobial peptides on the graphene-modified silk tattoo substrates. From dream to reality, our future connected tooth, although not yet a clinical reality, is driving the research of many curious people (Fig. 14). (50)

The future of personalized medicine is very real, we speak of "4 P's": Predictive, Preventive, Personalized and Participatory. It involves universal detection, generalized screening and treatment, if necessary, that is unique and adapted to the individual and his genetics. In this respect, all the major pharmaceutical companies have understood the interest of salivary tests and have started to collaborate with emerging web companies in this field. This futuristic evolution of tomorrow's medicine is based on the progress already made in genetics, pharmacology, nanotechnology and computer science. Each patient would be treated individually according to his or her genetic and environmental specificities.

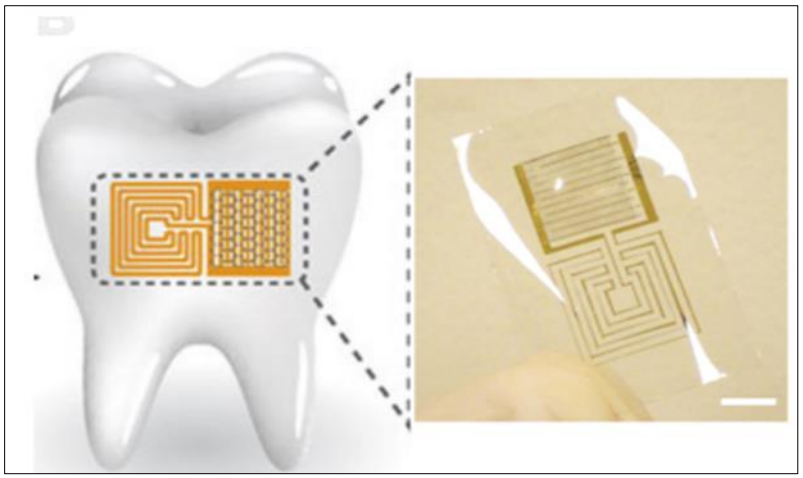


Figure 14 "Connected tooth" dental tattoo-based sensor for bacteria monitoring. (50)

5. Conclusion

Given their accuracy, efficacy, ease of use, and cost-effectiveness, salivary diagnostic tests have demonstrated applications in clinical and basic sciences. Furthermore, diagnostic techniques based on this attractive fluid have the potential to screen an entire population for a specific disease in a timely manner. However, much work remains to be done to integrate saliva-based diagnostics into the daily use of the Moroccan dentist. Saliva collection methods and biomarkers need to be standardized and validated. In addition, new tests and devices need to be developed at a commercially feasible pace. This may involve significant costs and may require a cooperative agreement among various stakeholders, including government, funding agencies, academia, and the private sector. Last but not least, these nontraditional saliva-based diagnostic tests would require widespread acceptance by insurance companies, dentists, and other health care professionals, for whom additional studies must demonstrate and establish their accuracy and cost-effectiveness. In conclusion, we anticipate that in the future, dentists will be able to diagnose and monitor therapeutic strategies for metabolic diseases such as diabetes and obesity, inflammation, infection, stress response, cancers, and oral diseases in individuals with only a drop of saliva within their hospital settings

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed

References

[1] Malamud D, Rodriguez-Chavez IR. Saliva as a Diagnostic Fluid. Dent Clin North Am. janv 2011;55(1):159-78.

[2] Pappa E, Vougas K, Zoidakis J, Vastardis H. Proteomic advances in salivary diagnostics. Biochim Biophys Acta BBA - Proteins Proteomics. 1 nov 2020;1868(11):140494.

[3] Roi A, Rusu LC, Roi CI, Luca RE, Boia S, Munteanu RI. A New Approach for the Diagnosis of Systemic and Oral Diseases Based on Salivary Biomolecules. Dis Markers. 17 févr 2019; 2019:8761860.

[4] Miller E, Sikes HD. Addressing barriers to the development and adoption of rapid diagnostic tests in global health. Nanobiomedicine. 2015 Jan 1;2:6.

[5] Wang C, Liu M, Wang Z, Li S, Deng Y, He N. Point-of-care diagnostics for infectious diseases: From methods to devices. Nano Today. avr 2021; 37:101092.

[6] Castagnola M, Scarano E, Passali GC, Messana I, Cabras T, Iavarone F, et al. Salivary biomarkers and proteomics: future diagnostic and clinical utilities. Acta Otorhinolaryngol Ital. avr 2017;37(2):94-101.

[7] Khurshid Z, Warsi I, Moin SF, Slowey PD, Latif M, Zohaib S, et al. Chapter Six - Biochemical analysis of oral fluids for disease detection. In: Makowski GS, éditeur. Advances in Clinical Chemistry [Internet]. Elsevier; 2021 [cité 6

juin 2021]. p. 205-53. Disponible sur: https://www.sciencedirect.com/science/article/pii/S0065242320300408

[8] Miller CS, Foley JD, Bailey AL, Campell CL, Humphries RL, Christodoulides N, et al. Current developments in salivary diagnostics. Biomark Med. févr 2010;4(1):171-89.

[9] Chojnowska S, Baran T, Wilińska I, Sienicka P, Cabaj-Wiater I, Knaś M. Human saliva as a diagnostic material. Adv Med Sci. 1 mars 2018;63(1):185-91.

[10] Baliga S, Muglikar S, Kale R. Salivary pH: A diagnostic biomarker. J Indian Soc Periodontol. 2013;17(4):461-5.

[11] Wang A, Wang CP, Tu M, Wong DTW. Oral Biofluid Biomarker Research: Current Status and Emerging Frontiers. Diagnostics [Internet]. 17 déc 2016 [cité 27 mai 2021];6(4). Disponible sur: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5192520/

[12] Khurshid Z, Zohaib S, Najeeb S, Zafar MS, Slowey PD, Almas K. Human Saliva Collection Devices for Proteomics: An Update. Int J Mol Sci. 6 juin 2016;17(6):846.

[13] Zhang A, Sun H, Wang X. Saliva Metabolomics Opens Door to Biomarker Discovery, Disease Diagnosis, and Treatment. Appl Biochem Biotechnol. nov 2012;168(6):1718-27.

[14] The Diagnostic Power of Saliva and its Impact on Dentistry and Beyond [Internet]. CareQuest Institute for Oral Health. [cité 23 nov 2021]. Disponible sur: https://www.carequest.org/education/webinars/diagnostic-power-saliva-and-its-impact-dentistry-and-beyond

[15] Dawes C, Wong DTW. Role of saliva and salivary diagnostics in advancing oral health. J Dent Res. 2019 Feb 1;98(2):133-41.

[16] Rathnayake N, Gieselmann DR, Heikkinen AM, Tervahartiala T, Sorsa T. Salivary Diagnostics—Point-of-Care diagnostics of MMP-8 in dentistry and medicine. Diagnostics. mars 2017;7(1):7.

[17] Slavkin HC, Fox CH, Meyer DM. Salivary diagnostics and its impact in dentistry, research, education, and the professional community. Adv Dent Res. oct 2011;23(4):381-6.

[18] Bin Mubayrik A, Al Dosary S, Alshawaf R, Alduweesh R, Alfurayh S, Alojaymi T, et al. Public Attitudes Toward Chairside Screening for Medical Conditions in Dental Settings. Patient Prefer Adherence. 2 févr 2021;15:187-95.

[19] Segal A, Wong DT. Salivary diagnostics: enhancing disease detection and making medicine better. Eur J Dent Educ Off J Assoc Dent Educ Eur. févr 2008;12 Suppl 1:22-9.

[20] Wren ME, Shirtcliff EA, Drury SS. Not All Biofluids Are Created Equal: Chewing Over Salivary Diagnostics and the Epigenome. Clin Ther. 1 mars 2015;37(3):529-39.

[21] Zhang Y, Sun J, Lin CC, Abemayor E, Wang MB, Wong DTW. The emerging landscape of salivary diagnostics. Periodontol 2000. févr 2016;70(1):38-52.

[22] Uchida H, Ovitt CE. Novel impacts of saliva with regard to oral health. J Prosthet Dent [Internet]. 14 juin 2021 [cité 1 oct 2021];0(0). Disponible sur: https://www.thejpd.org/article/S0022-3913(21)00273-0/fulltext

[23] Kubala E, Strzelecka P, Grzegocka M, Lietz-Kijak D, Gronwald H, Skomro P, et al. A Review of Selected Studies That Determine the Physical and Chemical Properties of Saliva in the Field of Dental Treatment. BioMed Res Int. 9 mai 2018;2018:6572381.

[24] Les tests salivaires [Internet]. Dentalespace. [cité 23 nov 2021]. Disponible sur: https://www.dentalespace.com/praticien/formationcontinue/test-salivaires/

[25] Javaid MA, Ahmed AS, Durand R, Tran SD. Saliva as a diagnostic tool for oral and systemic diseases. J Oral Biol Craniofacial Res. 1 janv 2016;6(1):67-76.

[26] Ghallab NA. Diagnostic potential and future directions of biomarkers in gingival crevicular fluid and saliva of periodontal diseases: Review of the current evidence. Arch Oral Biol. 1 mars 2018;87:115-24.

[27] Lee DYH, Wong DDT. Saliva: An emerging biofluid for early detection of diseases. Am J Dent. août 2009;22(4):241.

[28] 20210626_LIVRE__TEST_COVID.pdf [Internet]. [cité 23 oct 2021]. Disponible sur: https://www.acadpharm.org/dos_public/20210626_LIVRE__TEST_COVID.PDF

[29] Santosh TS, Parmar R, Anand H, Srikanth K, Saritha M. A Review of Salivary Diagnostics and Its Potential Implication in Detection of Covid-19. Cureus [Internet]. avr 2020 [cité 19 juin 2021];12(4). Disponible sur: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7164701/

[30] Goggolidou P, Hodges-Mameletzis I, Purewal S, Karakoula A, Warr T. Self-Testing as an Invaluable Tool in Fighting the COVID-19 Pandemic. J Prim Care Community Health. 29 sept 2021;12:21501327211047784.

[31] Boum Y, Eyangoh S, Okomo MC. Beyond COVID-19—will self-sampling and testing become the norm? Lancet Infect Dis. sept 2021;21(9):1194-5.

[32] Benda A, Zerajic L, Ankita A, Cleary E, Park Y, Pandey S. COVID-19 Testing and Diagnostics: A Review of Commercialized Technologies for Cost, Convenience and Quality of Tests. Sensors. janv 2021;21(19):6581.

[33] Kritikos A, Caruana G, Brouillet R, Miroz JP, Abed-Maillard S, Stieger G, et al. Sensitivity of Rapid Antigen Testing and RT-PCR Performed on Nasopharyngeal Swabs versus Saliva Samples in COVID-19 Hospitalized Patients: Results of a Prospective Comparative Trial (RESTART). Microorganisms. sept 2021;9(9):1910.

[34] Ulinici M, Covantev S, Wingfield-Digby J, Beloukas A, Mathioudakis AG, Corlateanu A. Screening, Diagnostic and Prognostic Tests for COVID-19: A Comprehensive Review. Life. juin 2021;11(6):561.

[35] Klein JAF, Krüger LJ, Tobian F, Gaeddert M, Lainati F, Schnitzler P, et al. Head-to-head performance comparison of self-collected nasal versus professional-collected nasopharyngeal swab for a WHO-listed SARS-CoV-2 antigen-detecting rapid diagnostic test. Med Microbiol Immunol (Berl). 2021;210(4):181-6.

[36] Malarvili MB, Alexie M, Dahari N, Kamarudin A. On Analyzing Capnogram as a Novel Method for Screening COVID-19: A Review on Assessment Methods for COVID-19. Life. oct 2021;11(10):1101.

[37] WHO-2019-nCoV-Antigen_Detection-2020.1-fre.pdf [Internet]. [cité 29 oct 2021]. Disponible sur: https://apps.who.int/iris/bitstream/handle/10665/334409/WHO-2019-nCoV-Antigen_Detection-2020.1-fre.pdf?sequence=1andisAllowed=y

[38] Corman VM, Haage VC, Bleicker T, Schmidt ML, Mühlemann B, Zuchowski M, et al. Comparison of seven commercial SARS-CoV-2 rapid Point-of-Care Antigen tests [Internet]. 2020 nov [cité 29 oct 2021] p. 2020.11.12.20230292. Disponible sur: https://www.medrxiv.org/content/10.1101/2020.11.12.20230292v1

[39] Dinnes J, Deeks JJ, Berhane S, Taylor M, Adriano A, Davenport C, et al. Rapid, point-of-care antigen and molecular-based tests for diagnosis of SARS-CoV-2 infection. Cochrane Database Syst Rev. 24 mars 2021;2021(3):CD013705.

[40] La Marca A, Capuzzo M, Paglia T, Roli L, Trenti T, Nelson SM. Testing for SARS-CoV-2 (COVID-19): a systematic review and clinical guide to molecular and serological in-vitro diagnostic assays. Reprod Biomed Online. 1 sept 2020;41(3):483-99.

[41] Klabbers RE, Muwonge TR, Ayikobua E, Izizinga D, Bassett IV, Kambugu A, et al. Health Worker Perspectives on Barriers and Facilitators of Assisted Partner Notification for HIV for Refugees and Ugandan Nationals: A Mixed Methods Study in West Nile Uganda. AIDS Behav. 1 oct 2021;25(10):3206-22.

[42] Tang MS, Hock KG, Logsdon NM, Hayes JE, Gronowski AM, Anderson NW, et al. Clinical Performance of Two SARS-CoV-2 Serologic Assays. Clin Chem. 1 août 2020;66(8):1055-62.

[43] Le Maroc va produire des tests Covid 19 [Internet]. Bled.news. 2021 [cité 29 oct 2021]. Disponible sur: https://bled.news/le-maroc-va-produire-des-tests-covid-19/

[44] Excluded from the sale and performance of Covid-19 tests, pharmacists demand their own ministry of pharmacy and medicine [Internet]. Hespress Français. 2021 [cited 11 Nov 2021]. Available at: https://fr.hespress.com/220770-exclus-de-la-vente-et-la-realisation-des-tests-les-pharmaciens-reclament-leur-propre-ministere.html

[45] Maroc: la vérité sur le retrait des tests salivaires des pharmacies [Internet]. Le Site Info. 2021 [cité 11 nov 2021]. Disponible sur: https://www.lesiteinfo.com/maroc/maroc-la-verite-sur-le-retrait-des-tests-salivaires-des-pharmacies/

[46] Corstjens PLAM, Abrams WR, Malamud D. Saliva and viral infections. Periodontol 2000. févr 2016;70(1):93-110.

[47] Corstjens PLAM, Abrams WR, Malamud D. Detecting viruses by using salivary diagnostics. J Am Dent Assoc 1939. oct 2012;143(10 0):12S-18S.

[48] Slowey PD. Saliva Collection Devices and Diagnostic Platforms. In: Streckfus CF, éditeur. Advances in Salivary Diagnostics [Internet]. Berlin, Heidelberg: Springer; 2015 [cité 1 nov 2021]. p. 33-61. Disponible sur: https://doi.org/10.1007/978-3-662-45399-5_3

[49] Derruau S, Robinet J, Untereiner V, Piot O, Sockalingum GD, Lorimier S. Vibrational Spectroscopy Saliva Profiling as Biometric Tool for Disease Diagnostics: A Systematic Literature Review. Molecules. 10 sept 2020;25(18):4142.

[50] Gao W, Brooks GA, Klonoff DC. Wearable physiological systems and technologies for metabolic monitoring. J Appl Physiol Bethesda Md 1985. 1 mars 2018;124(3):548-56.

[51] Pappa E, Kousvelari E, Vastardis H. Saliva in the "Omics" era: A promising tool in paediatrics. Oral Dis. 2019;25(1):16-25.

[52] Kim J, Campbell AS, de Ávila BEF, Wang J. Wearable biosensors for healthcare monitoring. Nat Biotechnol. avr 2019;37(4):389-406.

[53] Chhiba L, Zaher B, Mustapha S, Marzak A. Glucose Sensing for Diabetes Monitoring: From Invasive to Wearable Device. In 2020. p. 350-64.

[54] Du Y, Zhang W, Wang ML. An On-Chip Disposable Salivary Glucose Sensor for Diabetes Control. J Diabetes Sci Technol. 5 avr 2016;10(6):1344-52.

[55] Desai GS, Mathews ST. Saliva as a non-invasive diagnostic tool for inflammation and insulin-resistance. World J Diabetes. 15 déc 2014;5(6):730-8.

[56] Hartman ML, Goodson J, Barake R, Alsmadi O, Al-Mutawa S, Ariga J, et al. Salivary Biomarkers in Pediatric Metabolic Disease Research. Pediatr Endocrinol Rev PER. 1 mars 2016;13:602-11.

[57] Katsareli EA, Dedoussis GV. Biomarkers in the field of obesity and its related comorbidities. Expert Opin Ther Targets. avr 2014;18(4):385-401.

[58] Starzak DE, Konkol KF, McKune AJ. Effects of Cardiorespiratory Fitness and Obesity on Salivary Secretory IgA and Alpha-Amylase in South African Children. Children. sept 2016;3(3):12.

[59] Goodson JM, Kantarci A, Hartman ML, Denis GV, Stephens D, Hasturk H, et al. Metabolic disease risk in children by salivary biomarker analysis. PloS One. 2014;9(6):e98799.

[60] Melguizo-Rodríguez L, Costela-Ruiz VJ, Manzano-Moreno FJ, Ruiz C, Illescas-Montes R. Salivary Biomarkers and Their Application in the Diagnosis and Monitoring of the Most Common Oral Pathologies. Int J Mol Sci. janv 2020;21(14):5173.

[61] Kaczor-Urbanowicz KE, Martin Carreras-Presas C, Aro K, Tu M, Garcia-Godoy F, Wong DT. Saliva diagnostics – Current views and directions. Exp Biol Med. mars 2017;242(5):459-72.

[62] Zhang CZ, Cheng XQ, Li JY, Zhang P, Yi P, Xu X, et al. Saliva in the diagnosis of diseases. Int J Oral Sci. sept 2016;8(3):133-7.

[63] Gonçalves AC, Marson FA de L, Mendonça RM de H, Ribeiro JD, Ribeiro AF, Paschoal IA, et al. Saliva as a potential tool for cystic fibrosis diagnosis. Diagn Pathol. 19 mars 2013;8:46.

[64] Salivary Diagnostics, Current Reality and Future Prospects | IntechOpen [Internet]. [cité 8 nov 2021]. Disponible sur: https://www.intechopen.com/chapters/48033

[65] Mauch RM, Rossi CL, Nolasco da Silva MT, Bianchi Aiello T, Ribeiro JD, Ribeiro AF, et al. Secretory IgA-mediated immune response in saliva and early detection of Pseudomonas aeruginosa in the lower airways of pediatric cystic fibrosis patients. Med Microbiol Immunol (Berl). avr 2019;208(2):205-13.

[66] Nguyen TTH, Sodnom-Ish B, Choi SW, Jung HI, Cho J, Hwang I, et al. Salivary biomarkers in oral squamous cell carcinoma. J Korean Assoc Oral Maxillofac Surg. 2020;46(5):301-12.

[67] Arif S, Qudsia S, Urooj S, Chaudry N, Arshad A, Andleeb S. Blueprint of quartz crystal microbalance biosensor for early detection of breast cancer through salivary autoantibodies against ATP6AP1. Biosens Bioelectron. 15 mars 2015;65:62-70.

[68] Agha-Hosseini F, Mirzaii-Dizgah I, Rahimi A. Correlation of serum and salivary CA15-3 levels in patients with breast cancer. Med Oral Patol Oral Cirugia Bucal. 1 oct 2009;14(10):e521-524.

[69] Wang X, Kaczor-Urbanowicz KE, Wong DTW. Salivary Biomarkers in Cancer Detection. Med Oncol Northwood Lond Engl. janv 2017;34(1):7.

[70] Wong D. Salivary Diagnostics. Oper Dent. 1 oct 2012;37(6):562-70.

[71] Zhang L, Xiao H, Zhou H, Santiago S, Lee JM, Garon EB, et al. Development of transcriptomic biomarker signature in human saliva to detect lung cancer. Cell Mol Life Sci CMLS. oct 2012;69(19):3341-50.

[72] Zhang L, Farrell JJ, Zhou H, Elashoff D, Akin D, Park NH, et al. Salivary transcriptomic biomarkers for detection of resectable pancreatic cancer. Gastroenterology. mars 2010;138(3):949-957.e1-7.

[73] Wang X, Kaczor-Urbanowicz KE, Wong DTW. Salivary biomarkers in cancer detection. Med Oncol. 10 déc 2016;34(1):7.

[74] Giacomello G, Scholten A, Parr MK. Current methods for stress marker detection in saliva. J Pharm Biomed Anal. 30 nov 2020;191:113604.

[75] Chojnowska S, Ptaszyńska-Sarosiek I, Kępka A, Knaś M, Waszkiewicz N. Salivary Biomarkers of Stress, Anxiety and Depression. J Clin Med. janv 2021;10(3):517.

[76] van Veen JF, van Vliet IM, DeRijk RH, van Pelt J, Mertens B, Zitman FG. Elevated alpha-amylase but not cortisol in generalized social anxiety disorder. Psychoneuroendocrinology. 1 nov 2008;33(10):1313-21.

[77] Jafari A, Pouramir M, Shirzad A, Motallebnejad M, Bijani A, Moudi S, et al. Evaluation of Salivary Alpha Amylase as a Biomarker for Dental Anxiety. Iran J Psychiatry Behav Sci [Internet]. 31 mars 2018 [cité 17 nov 2021];12(1). Disponible sur: https://sites.kowsarpub.com/ijpbs/articles/9350.html#abstract

[78] Shetty V, Zigler C, Robles TF, Elashoff D, Yamaguchi M. DEVELOPMENTAL VALIDATION OF A POINT-OF-CARE, SALIVARY α-AMYLASE BIOSENSOR. Psychoneuroendocrinology. févr 2011;36(2):193-9.

[79] Kerémi B, Beck A, Fábián T, Fábián G, Szabo G, Nagy Á, et al. Stress and Salivary Glands. Curr Pharm Des. 15 févr 2017;23.

[80] Nobis A, Zalewski D, Waszkiewicz N. Peripheral Markers of Depression. J Clin Med. déc 2020;9(12):3793.

[81] Biringer E, Egeland J, Stordal KI, Lund A. P247 - The predictive value of saliva cortisol for remission of major depressive disorder. Eur Psychiatry. mars 2007;22(S1):S172-S172.

[82] VerOFy® and LIAM™ [Internet]. Oasis Diagnostics®. 2012 [cité 18 nov 2021]. Disponible sur: https://4saliva.com/products/verofy/

[83] Schwedt TJ. Chronic migraine. BMJ. 24 mars 2014;348:g1416.

[84] Jang MU, Park JW, Kho HS, Chung SC, Chung JW. Plasma and saliva levels of nerve growth factor and neuropeptides in chronic migraine patients. Oral Dis. 2011;17(2):187-93.

[85] Ruhl S. The scientific exploration of saliva in the post-proteomic era: from database back to basic function. Expert Rev Proteomics. févr 2012;9(1):85-96.

[86] Khan RS, Khurshid Z, Yahya Ibrahim Asiri F. Advancing Point-of-Care (PoC) Testing Using Human Saliva as Liquid Biopsy. Diagnostics. 4 juill 2017;7(3):39.

[87] Koneru S, Tanikonda R. Salivaomics - A promising future in early diagnosis of dental diseases. Dent Res J. 2014;11(1):11-5.

[88] Gug IT, Tertis M, Hosu O, Cristea C. Salivary biomarkers detection: Analytical and immunological methods overview. TrAC Trends Anal Chem. 1 avr 2019;113:301-16.

[89] Mannoor MS, Tao H, Clayton JD, Sengupta A, Kaplan DL, Naik RR, et al. Graphene-based wireless bacteria detection on tooth enamel. Nat Commun. 27 mars 2012;3(1):763.