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Saliva: A Versatile Diagnostic Tool for Health Assessment

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ABSTRACT:

Given the tiny concentration of salivary ingredients, saliva is being investigated as a diagnostic tool. For health practitioners, monitoring a patient's health and illness state through saliva is a highly important aim. Because it also satisfies the need for a low-cost, non-invasive, and user-friendly screening technique. In comparison to serum and other biological fluids, it provides a cost-effective and non-invasive means of collection for tracking the advancement of disease and overall health. Saliva is used in research not only for tooth health but also for a number of other systemic conditions. Molecular techniques are becoming more and more popular, which has led to their use as sensitive and specific biomarkers in transcriptomics, proteomics, and genomics.

Numerous elements present in these fluids can serve as biomarkers for the diagnosis of both local and systemic illnesses, including neurological, metabolic, cardiovascular, and cancer diseases. The fundamentals of salivary diagnostic expectoration techniques and their use in a variety of local and systemic illnesses are covered in this abstract. The importance of salivary biomarkers as a diagnostic tool has been highlighted. When anything is awry, the human body has a special way of telling you about it. Through promoting health and delaying the progression of sickness, the chemicals found in bodily fluids can aid in the early diagnosis of illnesses. Better healthcare is made possible by these biomarkers, which are attracting a lot of attention from researchers. .. The future belongs to biosensors that can identify these biomarkers, particularly Point of Care (POC) biosensors that do not require a patient to be physically present in the hospital. We still have a ways to go in applying biosensors for the detection of complex and systemic diseases. Among bodily fluids, saliva-based biosensors are becoming more and more popular because of their non-invasive collection method, capacity to detect systemic disorders, and ability to detect periodontal disease. Saliva-based diagnostic biosensors have garnered significant attention, since kits for ancestry prediction are being sent home by firms. Saliva-based testing for COVID-19 has demonstrated the utility of the inexpensive collection and its successful clinical application. The identification of systemic disorders is a rapidly expanding field of research, based on universal biomarkers. Saliva-based biosensors have been extensively studied, however as POC devices, they still have drawbacks and restrictions. This review discusses the value of saliva and how effective it is as a biosensor. It also includes suggestions that should be taken into account in order to make it usable as a potential diagnostic tool.

KEYWORDS

Saliva, Diagnostic tool, Biomarkers, Non-invasive Systemic disorders, Biosensors Point of Care, COVID-19 testing, Ancestry prediction Clinical application.

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Introduction

Human diseases having global impact include cancer, cardiovascular, metabolic, and neurological diseases. Saliva is a unique

biological dynamic fluid that has varying spectrum of proteins, polypeptides, nucleic acids, electrolytes, and hormones. It is an exocrine secretion of the salivary glands which is hypotonic in nature with a PH of 7.2-7.4. Early disease detection is essential for appropriate treatment and complete eradication of any disease that diverges from a state of



healthy well-being. Because saliva is simple to obtain, inexpensive, easily stored, and non-invasive, saliva-based diagnosis has attracted a lot of interest. There are collection problems with other bodily fluids including blood and urine that are frequently utilized to diagnose diseases. Biocomponents found in saliva may be useful as biomarkers. Saliva will therefore complement the diagnostic toolkit by offering vital details regarding systemic and oral health. Salivary biomarkers span a variety of omic domains, including transcriptomics, metabolomics, and proteomics. This study highlights saliva's potential as a diagnostic specimen by examining multiple research in saliva-related diagnostics and their relationship to systemic disorders. Saliva's high accessibility places it at the forefront of biomarker research. Saliva has been considered a practical and affordable test for coronavirus disease 2019 (COVID-19), particularly during the pandemic.(1,2). One essential bodily fluid that keeps the mouth cavity free from infections is saliva (3). It controls the enzymatic activity in the mouth and functions as a lubricant to safeguard teeth. A decrease in salivary flow may be a sign of dental caries risk, according to research (4). The salivary biofilm naturally shields the tooth from erosion and decay by covering the surface of the tooth (5). Saliva is secreted by the major and minor salivary glands, travels through the ducts, and ends up in the mouth (6). The parotid gland, submandibular gland, and sublingual gland are important glands that help secrete saliva. They are made up of both serous and mucous cells. Salivary composition is influenced by diet and nutrient intake. The presence of genetic material in a person's saliva facilitates several genomic applications, including forensic evidence (7). Saliva contains proteins such as amylases and immunoglobulin, which break down starch and other compounds to help digestion (8). It also contains antibacterial components that help to keep the oral microbiome healthy, as well as chemicals like urea and ammonia (9). Together, these components in saliva make it a potent and effective diagnostic tool. As a result, there have been notable advancements in saliva testing, such as HIV testing as an indicator of acquired immunodeficiency syndrome (AIDS) and HPV detection for HNSCC. Furthermore, myocardial

enzyme testing for myocardial infarction monitoring and cortisol for stress and inflammatory status detection remind us of the use of saliva in human-derived biomarker research.(10)

Surprisingly, a study revealed that couples who shared saliva—for example, by sipping juice via the same straw—had closer relationships.(11) Women who have been assaulted by an intimate partner, on the other hand, show measurable cortisol level changes in their saliva.(12) This demonstrates the significance of saliva in psychological studies. These research expand our basic understanding of saliva. Saliva is becoming increasingly essential in health issues.

Unlocking Saliva's Potential

Saliva has emerged as a viable diagnostic tool since it is non-invasive, inexpensive, and simple to collect. It provides a pleasant and user-friendly alternative to traditional blood and tissue sample procedures, making it appropriate for routine monitoring and screening. Saliva has a diverse set of biomarkers that represent overall health, enabling for the identification of a variety of diseases and ailments. Challenges include lower biomarker concentrations, variable composition due to nutrition and hydration, and limited diagnostic breadth compared to blood or tissue samples (13). Additional study stresses saliva's potential as a diagnostic medium, notably in discovering biomarkers for oral and systemic disorders. The study emphasizes the necessity for consistent collection and analysis techniques to improve the reliability and accuracy of saliva-based diagnostic assays (14). Furthermore, advances in saliva-based biosensors have enabled quick and point-of-care testing for a variety of health issues. However, they also point up problems associated to technology adaption and the need for continual increases in sensor sensitivity and specificity (15). Despite these challenges, saliva-based diagnostics show potential for increasing healthcare accessibility and early illness identification.

Saliva can act as a buffer in the oral cavity, preventing viruses from infecting it, as well as neutralizing the acids created by very acidic foods (16). This keeps the enamel from



deteriorating and protects the teeth. Consuming fermented carbohydrates in cookies, soda, and sweets, for example, can cause a drop in oral pH, and salivary peptides are generated to elevate saliva pH in order to maintain normal oral pH (17). Ammonia and urea in saliva raise pH, allowing the buffer system to function properly. The capacity to detect normal vs. abnormal amounts of urea and other compounds in saliva opens the way for varied biosensor development. Specific biosensors may test for various salivary biomarkers and categorize them as normal vs abnormal. This analysis can be used to detect infections or diagnose potential diseases based on aberrant signs or changes in the levels of important enzymes or biomarkers.

Saliva's fluid properties and constituent parts

enable it to perform additional tasks such as washing, solubilizing food, removing microorganisms, lubricating soft tissues, forming boluses, swallowing, speaking, and facilitating mastication. Saliva components also aid in digestion, mucosal coating, and antibacterial defense (18). An average person can produce 600 mL of saliva per day. Saliva contains 99% water and one percent organic molecules such as salivary amylase, mucopolysaccharide, mucin, and lysozymes, and some inorganic matter such as sodium, potassium, calcium chloride, and thiocyanate ions. Various bio-components are also found in saliva, including protein and related molecules, nucleic acid components, and endogenous and exogenous metabolites (19)



FIG .1: Biocomponents detectable in saliva (20,21)

Saliva's chemical makeup and ratios are dynamic with physiological state. Changes in diet, mental health, and disease can all affect the makeup of saliva. The way that diseases alter the composition of saliva makes it easier to use

saliva in clinical research for purposes including prognosis, therapy, and diagnosis.

It mainly addresses the proceedings to shed light on the recent promising usage of saliva testing in clinical diagnostics.

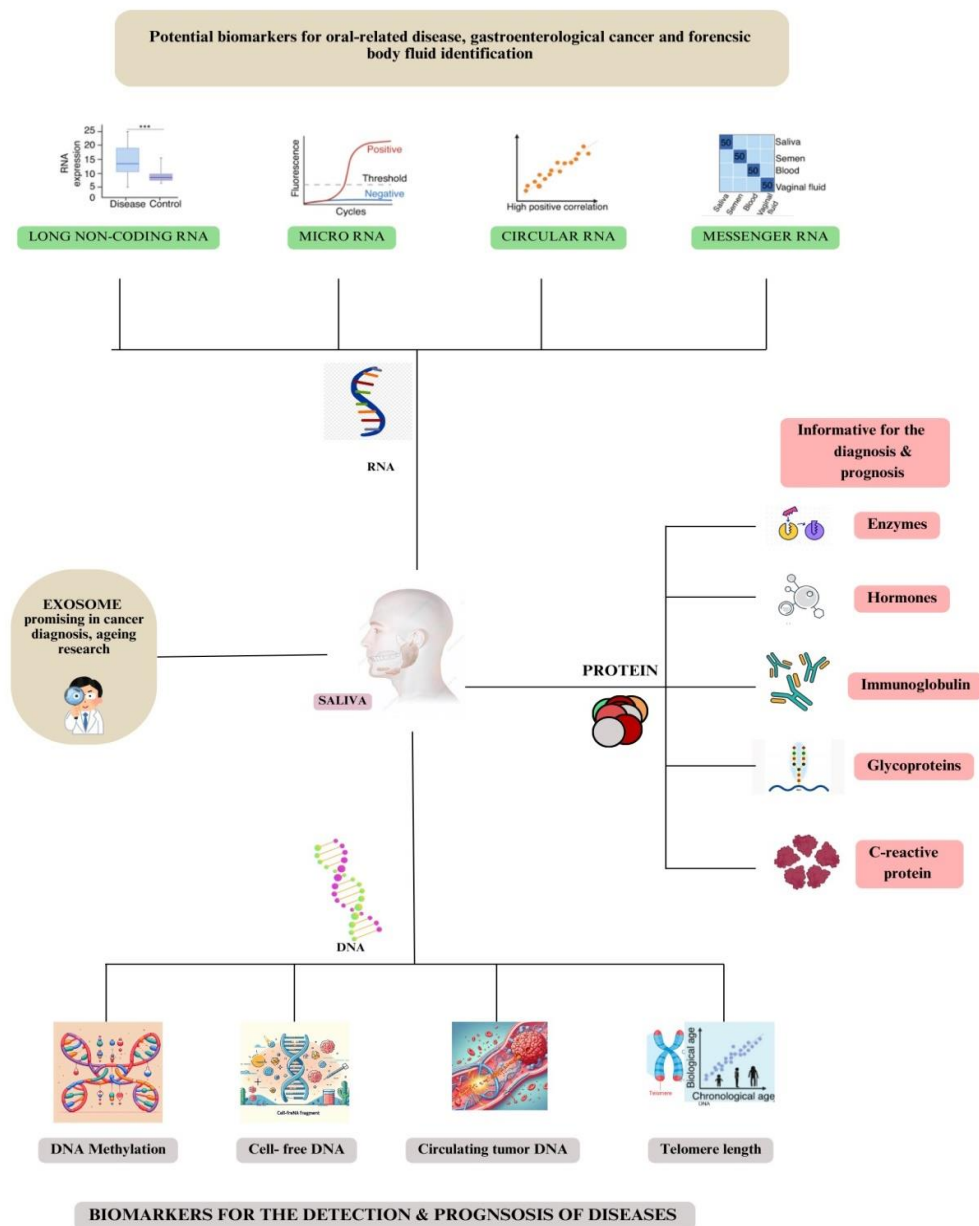


Fig 2: Exploring the Potential of Saliva Biomarker Testing in Clinical Diagnostics

Biosensor Selections:

Biosensors are an exciting addition to the world of nanomedicine and technology. They allow patients and healthcare providers to monitor disease progressions and treatment efficiency

(22). This play a vital role in detecting diseases early on and surveilling any crucial data to provide patients with early treatments and prevent disease progression.



Biosensors	Main Principle	Advantages	Disadvantages	References
Titanium Biosensor	Utilizing titanium coated biosensors to gain insight on periodontal health and detecting harmful levels of streptococcus Gordonii. This will allow for further testing on autoimmune diseases and biomarkers present pre and post surgeries	Noninvasive Utilizes the understanding of a bacteria that has been the subject of extensive research—Allows CHX, which is used widely in hospital/surgical settings, to be further utilized as a protective oral health tool – Titanium is durable, does not corrode, high quality	- Using CHX as a coating may introduce other factors not accounted for - May be costly to obtain all the materials necessary for this biosensor	[85]
Alpha-Amylase Biosensor (Fig. 4)	- Detecting levels of alphaamylase in saliva	- Readily available - Noninvasive - Provides insight on the diet of an individual—Concrete substance to test and manipulate that is constantly present in saliva - Provides insight on levels of acidity in the oral cavity due to digestion of complex carbohydrates	- Diet of an individual may cause skewed data - Pre-existing health conditions may influence patient's results	[86]
Urea Smartphone Biosensor (Fig. 4)	- Detecting levels of salivary urea and connecting a transducer with a smartphone application that evaluates the range of urea levels (Fig. 3)	- Concrete substance to test and manipulate—Previous research articles on levels of urea show that higher levels of urea were associated with kidney disease or periodontal disease	- Requires the presence of a smartphone and most likely a WIFI connection to transfer data to clinics and medical professionals—Color detection may be skewed based on lighting and other environmental factors –May cause anxiety for patients who may panic if numbers are not within the same range	[87]
Non-invasive Impedimetric Biosensor for Oral Cancer Detection (Fig. 4)	- Efficient biomarker focused on detecting the cancer biomarker CYFRA-21-1 in saliva	- Noninvasive - Allows for early detection of cancer without any surgical procedures or costly treatments—Utilizes ELISA, a method that has been used vastly and can be manipulated by scientists - Introduces an extremely sensitive biosensor that detects precise values - IR spectroscopy bands utilized to detect specific functional groups	-May provide insight on lung cancer and or other cancers that may not be applicable to oral cancer - Requires clear understanding of CYFRA21-1 biomarker and its functionalization	



Salivary Biomarkers and Their Significance:

Saliva is a non-invasive diagnostic fluid that has drawn a lot of interest for the detection of cancer and other disorders. It has a wide range of biomarkers that can provide important information about the physiological and pathological state of an individual. Urea, gingival crevicular fluid (GCF), microbiota, and alpha-amylase are among the salivary biomarkers that show promise in the identification and tracking of cancer. Saliva contains a variety of biomarkers, some of which can be detected via infrared spectroscopy. Cancers can spread swiftly because cells multiply much more quickly. Saliva has been utilized as a diagnostic tool not only for periodontal disease surveillance (27, 28), but also for systemic inflammation identification and screening in epidemiological investigations (29). Researchers believe that biomarkers in saliva can signal tumors and offer alternatives for early diagnosis because they are found in cancer-detection biomolecules (30).

1. Urea as a Salivary Biomarker:

Urea is a waste product of protein metabolism that is excreted through urine and saliva. Elevated levels of salivary urea have been associated with various cancers, including oral cancer, breast cancer, and lung cancer (31, 32). The increased levels of urea in saliva may result from metabolic disturbances caused by the tumor or the body's response to the disease (33).

2. Gingival Crevicular Fluid (GCF) as a Salivary Biomarker:

GCF is a site-specific inflammatory exudate that can be collected non-invasively from the gingival sulcus. It contains a wide range of biomarkers, including proteins, enzymes, and cytokines, which can provide valuable information about the host's immune response and the presence of oral diseases, including oral cancer (34). Several studies have demonstrated the potential of GCF biomarkers, such as interleukin-6 (IL-6), interleukin-8 (IL-8), and matrix metalloproteinases (MMPs), in the early detection and monitoring of oral cancer (35, 36).

3. Microbiome as a Salivary Biomarker:

The human oral cavity harbors a diverse and complex microbial community, collectively known as the oral microbiome. Dysbiosis, or an imbalance in the oral microbiome, has been associated with various diseases, including oral cancer (37). Certain bacterial species, such as *Fusobacterium nucleatum* and *Porphyromonas gingivalis*, have been found to be enriched in oral cancer patients, suggesting their potential as salivary biomarkers (38, 39). The analysis of the salivary microbiome could provide valuable insights into the early detection and monitoring of oral cancer.

4. Alpha-Amylase as a Salivary Biomarker:

Alpha-amylase is an enzyme produced by the salivary glands and is involved in the digestion of starch. Elevated levels of salivary alpha-amylase have been observed in various types of cancer, including breast cancer, lung cancer, and ovarian cancer (40, 41). The increased levels of alpha-amylase may be related to the stress response associated with cancer or the tumor's metabolic activity (42).

These salivary biomarkers hold great promise for non-invasive cancer detection and monitoring. However, further research is needed to validate their clinical utility and establish standardized protocols for sample collection, processing, and analysis. The integration of multiple salivary biomarkers, along with advanced analytical techniques, may provide a more comprehensive and accurate approach to cancer diagnosis and management.

Saliva-Based Systemic Disease Diagnosis:

Saliva has shown promise as a diagnostic fluid that can be used to detect and track a number of systemic disorders. Its non-invasiveness, simplicity of collection, and abundance of biomarkers make it a compelling substitute for conventional diagnostic techniques. Salivary biomarkers have been shown in numerous studies to be useful in the detection of viral diseases, malignant tumors, inflammatory disorders, and metabolic diseases.

Metabolic Diseases: a. Heart Diseases: It has been discovered that saliva contains biomarkers for cardiovascular conditions, including cardiac



troponins, myoglobin, and C-reactive protein (CRP) (43,44). These biomarkers may help in the early diagnosis and follow-up of cardiac disorders.

Diabetes: It has been demonstrated that in individuals with diabetes, salivary glucose, insulin, and other metabolic indicators correlate with blood glucose levels (45,46). For diabetics, salivary analysis may offer a non-invasive substitute for blood glucose monitoring.

Liver disorders: Patients with liver disorders, such as viral hepatitis and cirrhosis, have been shown to have elevated levels of salivary enzymes, such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyltransferase (GGT) (47,48). These biomarkers may be useful in the detection and tracking of liver diseases.

Rheumatoid arthritis, an inflammatory disease:

Patients with rheumatoid arthritis have been reported to have higher levels of salivary biomarkers, including cytokines (e.g., interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF- α), and matrix metalloproteinases (MMPs) (49,50). These biomarkers may help with the disease's diagnosis and follow-up by offering insights into the inflammatory processes connected to it.

Malignant Tumors: a. Breast Cancer: Biomarkers for breast cancer, such as c-erbB-2, EGFR, and CA 15-3, have been found to be detectable by saliva (51,52). These biomarkers may be useful for tracking the development of breast cancer and for early detection.

Cystic Fibrosis: Patients with cystic fibrosis have been discovered to have altered salivary biomarkers, including mucins, cytokines, and proteases (53, 54). This hereditary condition may be easier to diagnose and track with salivary analysis.

Infectious Diseases: Antibodies, proteins, and other biomarkers found in saliva can be signs of bacterial infections, HIV, and hepatitis, among other infectious diseases (55,56). In environments

with limited resources, salivary diagnostics may offer a non-invasive substitute for the identification and tracking of these infections.

Inflammatory Bodies: a. Sjögren's Syndrome: Patients with Sjögren's syndrome have been reported to have higher levels of salivary biomarkers, including rheumatoid factor (RF), anti-Sjögren's syndrome-related antigen A (anti-SSA/Ro), and anti-Sjögren's syndrome-related antigen B (anti-SSB/La) (57,58). This autoimmune disease mostly affects the lacrimal and salivary glands, and these biomarkers can help with diagnosis and follow-up.

Multiple Sclerosis: It has been discovered that patients with multiple sclerosis have changed salivary biomarkers, such as oxidative stress markers, tumor necrosis factor-alpha (TNF- α), and cytokines (e.g., interleukin-6, IL-6) (59,60). For some patients, salivary analysis may offer a non-invasive way to track the course and intensity of their disease.

Bone Turnover Markers: Studies have looked into saliva as a possible source for finding these markers, which are helpful in the detection and tracking of bone disorders including osteoporosis. Bone-specific alkaline phosphatase (BAP), tartrate-resistant acid phosphatase (TRAP), and alkaline phosphatase (ALP) are salivary biomarkers that have been shown to correlate with bone mineral density and blood levels (61, 62). For evaluating bone health, salivary measurement of these indicators may provide a non-invasive substitute for serum-based testing.

Although salivary diagnostics seem very promising, more research is necessary to address potential confounding factors, validate biomarker specificity and sensitivity, and establish standardized methodologies. Furthermore, the accuracy and dependability of salivary diagnostics for a range of systemic disorders may be enhanced by the integration of several salivary biomarkers with cutting-edge analytical methods.

Periodontal diseases and dental caries:

Saliva also has its use in monitoring the level of Oral bacteria in it. Increased numbers of



Streptococcus mutants and lactobacilli in saliva have been associated with increased caries prevalence and root caries. Periodontal diseases have been associated with increased levels of aspartate Aminotransferase (AST) and alkaline phosphatase (ALP). Salivary AST can be used as a marker for monitoring periodontal disease. Lower levels of uric acid and albumin in the saliva were associated with periodontitis and diabetes. This could be attributed to the oxidative stress present in the oral cavity.

Maintaining oral health is vital, and the composition of saliva can provide important information about the onset and course of periodontal and dental caries illnesses. Numerous salivary biomarkers have been found to be possible predictors of these oral health issues.

Dental Caries:

Salivary Flow Rate: Because saliva is essential for remineralization and tooth enamel protection, a lower salivary flow rate is linked to a higher risk of dental caries (63). **Salivary Buffering Capacity:** Saliva has the ability to neutralize acid produced by cariogenic bacteria, which lowers the incidence of dental caries. This capacity is affected by concentrations of bicarbonate and phosphate (64). **Salivary Antimicrobial Proteins:** Saliva contains proteins with antimicrobial qualities that can stop the growth of cariogenic bacteria like Streptococcus mutans, including lactoferrin, lysozyme, and histatins (65). **Salivary calcium and phosphate:** These ions have a role in the formation of dental caries and are necessary for the remineralization of tooth enamel (66).

Periodontal disorders:

a. **Inflammatory Biomarkers:** Patients with periodontal disorders have been reported to have higher salivary levels of inflammatory biomarkers, such as interleukin-1 β (IL-1 β), interleukin-6 (IL-6), and matrix metalloproteinases (MMPs) (67).

b. **Oxidative Stress Markers:** Tissue degradation and periodontal inflammation have been linked to elevated levels of salivary oxidative stress markers, including malondialdehyde (MDA) and 8-hydroxydeoxyguanosine (8-OHdG) (68).

c. **Bone Turnover Markers:** Alkaline phosphatase

(ALP) and pyridinoline cross-linked carboxyterminal telopeptide of type I collagen (ICTP), two salivary bone turnover markers, can be used to detect loss of alveolar bone in periodontal disorders (69).

d. **Microbial biomarkers:** Saliva contains some periodontal pathogens, such as Porphyromonas gingivalis, Tannerella forsythia, and Treponema denticola, which can be used as biomarkers for the detection and tracking of periodontal disease (70).

A non-invasive method for the early identification, tracking, and risk assessment of periodontal and dental caries is provided by salivary diagnostics. To create standardized procedures, confirm the specificity and sensitivity of biomarkers, and deal with any confounding variables, more study is necessary.

Advantages of Oral Biosensors:

Oral biosensors, which utilize salivary biomarkers for disease detection and monitoring, offer several advantages over traditional diagnostic methods. These advantages have sparked significant interest in the development and application of oral biosensors in various fields, including healthcare, research, and personalized medicine. Here are some key advantages of oral biosensors:

Non-invasive and user-friendly: Oral biosensors rely on the collection of saliva, which is a non-invasive and painless procedure. This eliminates the need for invasive techniques like blood draws, making the process more comfortable and convenient for patients, especially for those requiring frequent monitoring, such as diabetic individuals or patients undergoing cancer treatment (71).

Real-time monitoring: Oral biosensors can provide real-time monitoring of physiological and pathological conditions by continuously analyzing salivary biomarkers. This allows for timely interventions and personalized treatment adjustments, potentially improving patient outcomes (72).

Cost-effective: Compared to traditional diagnostic methods that often involve expensive



equipment and specialized personnel, oral biosensors have the potential to be more cost-effective. The non-invasive nature of saliva collection and the possibility of developing point-of-care devices make oral biosensors a promising alternative for widespread implementation, especially in resource-limited settings (73).

Early disease detection: Salivary biomarkers can reflect systemic and oral health conditions at an early stage, enabling early disease detection and timely intervention. This can improve treatment outcomes and potentially reduce healthcare costs associated with advanced disease stages (74).

Monitoring treatment response: Oral biosensors can be used to monitor the effectiveness of therapeutic interventions by tracking changes in salivary biomarker levels. This information can guide treatment adjustments and personalized care, leading to improved patient outcomes (75).

Longitudinal monitoring: The non-invasive nature of saliva collection allows for repeated and longitudinal monitoring of biomarkers, enabling the tracking of disease progression or remission over time without causing significant discomfort to patients (76).

While oral biosensors hold great potential, ongoing research is necessary to address challenges such as biomarker validation, sensor sensitivity and specificity, and the development of robust and user-friendly devices. Continued advancements in this field could revolutionize disease diagnosis, monitoring, and personalized healthcare.

Saliva's Present Situation and Potential Future Use as a Diagnostic Tool:

Saliva has gained a lot of interest lately for its potential in illness monitoring and diagnosis, making it a viable diagnostic fluid. While the concept of using saliva as a diagnostic tool is not new, current technological developments and a better understanding of salivary biomarkers have sped up the process of developing and applying it in a number of sectors.

Salivary diagnostics is currently being extensively investigated for oral disease detection and surveillance, including dental caries, periodontal disorders, and oral cancer (77).

Salivary biomarkers have also been shown in many studies to have potential in the diagnosis and treatment of systemic problems, such as cancer, autoimmune diseases, cardiovascular diseases, and metabolic disorders (78,79).

Even with these developments, there are still a number of obstacles standing in the way of salivary diagnostics' widespread use. In order to guarantee accurate and repeatable results, standardization of saliva collection, processing, and analysis techniques is essential (80). Accurate interpretation also depends on setting reference ranges for salivary biomarkers and taking into consideration potential confounding variables such as circadian rhythms, pharmaceutical use, and environmental factors (81).

Future developments in salivary diagnostics will focus on creating point-of-care instruments and integrated biosensor platforms that can identify several indicators at once (82). These developments may completely change how diseases are diagnosed and tracked, opening the door to tailored and precision medical treatments. Additionally, there is a lot of potential to improve sensitivity, specificity, and automation by combining salivary diagnostics with cutting-edge technologies like nanotechnology, microfluidics, and artificial intelligence (83).

The main goals of current research are to validate salivary biomarkers, find new biomarkers, and create practical, affordable diagnostic instruments. Bringing salivary diagnostics from bench to bedside requires interdisciplinary collaborations between researchers, clinicians, and industrial partners (84).

Conclusion:

Because saliva is rich in biomarkers and is non-invasive, it has become a viable diagnostic fluid. Even though there has been a lot of progress, there are still issues with standardizing procedures for salivary biomarker collection, processing, and analysis as well as defining reference ranges (85). The development of point-of-care instruments and integrated biosensor platforms with simultaneous multi-biomarker detection capabilities is where the future is headed. In order to implement salivary



diagnostics in clinical settings and provide non-invasive, easily accessible, and reasonably priced methods for illness identification, tracking, and individualized treatment plans, interdisciplinary collaborations are essential. The future direction of salivary diagnostics lies in the development of point-of-care devices and integrated biosensor platforms capable of simultaneously detecting multiple biomarkers. These advancements could revolutionize disease diagnosis and monitoring, enabling personalized and precision medicine approaches. Moreover, the integration of salivary diagnostics with emerging technologies, such as micro fluidics, nanotechnology, and artificial intelligence, holds great promise for enhancing sensitivity, specificity, and automation. As salivary diagnostics develops further, it has the potential to revolutionize healthcare by offering accessible, affordable, non-invasive methods for illness detection, tracking, and individualized treatment plans.

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