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Review

Salivary Biomarkers Identification: Advances in Standard and Emerging Technologies

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Abstract: Introduction: Salivary biomarkers have been extensively studied in relation to oral disease, such as periodontal disease, oral cancer, and dental caries, as well as systemic conditions including diabetes, cardiovascular diseases, and neurological disorders. Literature Review: A systematic literature review was conducted, analyzing recent advancements in salivary biomarker research. Databases such as PubMed, Scopus, and Web of Science were searched for relevant studies published in the last decade. The selection criteria included studies focusing on the identification, validation, and clinical application of salivary biomarkers in diagnosing oral and systemic diseases. Various detection techniques, including enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR), mass spectrometry, and biosensor technologies, were reviewed to assess their effectiveness in biomarker analysis. Specific biomarkers, such as inflammatory cytokines, oxidative stress markers, and microRNAs, have been identified as reliable indicators of disease progression. Current Trends and Future Perspectives: Advances in proteomics, genomics, and metabolomics have significantly enhanced the ability to analyze salivary biomarkers with high sensitivity and specificity. Despite the promising findings, challenges remain in standardizing sample collection, processing, and analysis to ensure reproducibility and clinical applicability. Conclusions: Future research should focus on developing point-of-care diagnostic tools and integrating artificial intelligence to improve the predictive accuracy of salivary biomarkers.

Keywords: salivary biomarkers; oral diagnosis; biosensors; personalized medicine; artificial intelligence

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1. Introduction

Saliva has long been recognized as an essential biological fluid with critical roles in maintaining oral and systemic health [1]. Composed of water, electrolytes, proteins, enzymes, and nucleic acids, saliva serves multiple functions, including lubrication, digestion, immune defense, and antimicrobial activity [2]. Saliva can also be used to identify contaminants. In this context, "contaminants" refer to biological substances present in saliva that

may interfere with biomarker analysis. These include epithelial cells, gingival crevicular fluid, food debris, and microbial components. While epithelial cells and gingival crevicular fluids contain important biomarkers, their excessive presence can alter biomarker concentration levels and affect assay reliability. Proper saliva collection, processing, and analytical techniques are necessary to minimize unwanted interference and ensure reproducibility in biomarker detection. Salivary and serum concentrations of some compounds are closely related, and proteins and other molecules can enter saliva via the blood flow [3]. Salivary glands are extensively vascularized and can obtain chemicals from the circulation by active transport or passive diffusion.

Regarding this matter, it has been stated in recent research that saliva may be used as a diagnostic tool for detecting metabolic problems, endocrinological issues, systemic and local inflammation, and cardiovascular diseases (CVD) [4]. Since saliva collection is non-invasive and painless, it is recommended that it be used as an alternate diagnostic method [5]. Consequently, the ability to identify and measure biomarkers in salivary samples is incredibly appealing for research, clinical and discreet proactive healthcare applications aimed at early diagnosis of chronic diseases, enabling ongoing monitoring of those conditions [6]. Nevertheless, it is important to mention that certain biomarkers found in saliva cannot be used to diagnose a specific disease; instead, they may be utilized for a wide range of illnesses. Consequently, for a considerably more accurate diagnosis and prognosis, it is essential to take into account the many biomarkers impacted by each disease [7,8].

The study of salivary biomarkers has expanded significantly due to their ability to provide valuable insights into both oral and systemic diseases, making them a promising tool for early diagnosis, disease monitoring, and personalized medicine [9,10].

In light of this, the purpose of this review is to go over the pros and cons of using modern methods to find biomarkers in saliva that could be used for the diagnosis and treatment of various inflammatory chronic diseases, with the hope of eventually replacing current methods with detectable soluble mediators in saliva.

2. Literature Review

Salivary biocomponent presence was the basis for this research. Salivary biomarkers have the potential to be exploited for early identification and diagnosis of oral health issues and systemic disorders.

Biomarkers are measurable indicators of physiological and pathological processes, commonly used to diagnose and monitor various conditions. Traditionally, biomarkers have been analyzed in blood, urine, and tissue biopsies, which, although effective, present challenges related to patient discomfort, procedural risks, and high costs [11].

With advancements in biomedical research, alternative biofluids such as saliva are gaining attention as non-invasive sources of biomarkers. However, saliva offers several advantages over these conventional methods due to its non-invasive nature, ease of collection, and potential for real-time disease monitoring as illustrated in Figure 1 [12].

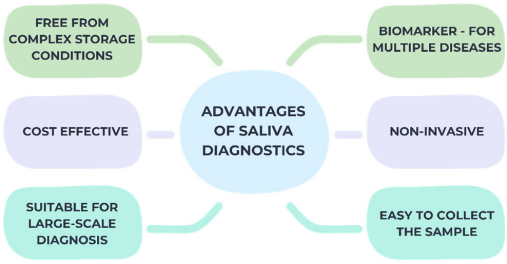


Figure 1. Advantages of salivary diagnostics.

2.1. Material and Method

The primary research question guiding this review was" What are the current methodologies used for salivary biomarker identification, and how have they evolved over time?"

A literature search was conducted across PubMed, Web of Science, ScienceDirect, Scopus, Google Scholar, MDPI, and Wiley Online Library, using keywords such as "salivary biomarkers", "biosensor technology", and "artificial intelligence". No date restrictions were applied. Data extraction focused on biomarker types, detection techniques, and clinical applicability. As a literature review, no ethical approval was required. Limitations include potential selection bias and data availability.

Research has demonstrated that salivary biomarkers can provide valuable insights into various physiological and pathological processes. Their potential extends beyond oral health, offering applications in detecting infections, monitoring metabolic disorders, and identifying inflammatory or autoimmune diseases [13]. Advances in salivary diagnostics leverage these components to develop biomarker panels for early detection of diseases, such as cancer, autoimmune disorders, and infectious diseases, improving personalized medicine and patient outcomes [14,15].

2.2. Types of Salivary Biomarkers

The biochemical composition and functional activity of salivary biomarkers dictate their categorization into several groups relevant to disease pathophysiology. Among the most common kinds of salivary biomarkers are the following:

Proteins and Enzymes—The wide variety of proteins and enzymes found in saliva is indicative of both normal and abnormal bodily functions. Inflammatory and autoimmune disorders are frequently linked to inflammatory cytokines like tumor necrosis factor-alpha TNF-α and interleukin-6 (IL-6) [16]. Periodontal disease research also often focusses on enzymes like matrix metalloproteinases (MMPs), which are important in tissue destruction [17]. The presence of about 2000 proteins in human saliva, with over 25% of those proteins being detected in human blood, highlights the significance of saliva chemistry as a potential tool for illness diagnosis and therapy monitoring [18].

DNA and RNA as genetic markers—Messenger RNA (mRNA), microRNA (miRNA), and circulating DNA are all types of nucleic acids found in saliva that may be able to indicate the presence of cancer or genetic abnormalities [19]. Some microRNAs have demonstrated potential as diagnostic tools for systemic disorders, such as diabetes and cardiovascular problems, while changes in salivary DNA methylation patterns have been associated with oral squamous cell carcinoma [20,21].

Metabolites—Changes in the metabolic makeup of saliva are indicative of metabolic processes occuring both locally and across the body. For example, individuals with diabetes tend to have higher amounts of glucose in their saliva, whereas oxidative stress indicators like malonedialdehyde (MDA) show that there is more oxidative damage in a number of chronic conditions [22].

While certain salivary biomarkers are valuable indicators of disease, many lack specificity for a single condition [23]. Biomarkers, such as C-reactive protein (CRP), interleukins (IL-6, IL-8), and matrix metalloproteinases (MMPs), are commonly associated with inflammatory responses and can be detected in multiple diseases, including periodontitis, systemic inflammatory disorders, and oral cancer [24–26]. This overlap limits the diagnostic utility of single biomarkers, necessitating a multi-biomarker panel approach to improve specificity and sensitivity.

Recent studies have demonstrated that combining proteomic, transcriptomic, and metabolomic biomarker panels provides a more accurate distinction between conditions with overlapping inflammatory profiles [27]. For example, oral squamous cell carcinoma

(OSCC) and chronic periodontitis share common inflammatory mediators, but the integration of multiple biomarkers improves disease classification and risk assessment [28]. Additionally, advancements in machine learning algorithms have facilitated the development of predictive models that analyze biomarker interactions, further enhancing diagnostic precision [29].

By integrating multi-biomarker panels, clinicians can achieve higher accuracy in disease detection and progression monitoring, paving the way for personalized diagnostic strategies in salivary biomarker research.

Microbiome Biomarkers—Salivary microbiota analysis has gained attention in recent years for its potential in diagnosing diseases. Dysbiosis, or imbalances in microbial composition, has been linked to periodontal disease, oral cancer, and even gastrointestinal disorders [30,31]. By studying shifts in bacterial populations, researchers can gain valuable insights into disease etiology and progression [32].

Although salivary biomarkers are increasingly studied in the context of diseases such as periodontitis, oral cancer, and systemic conditions, most investigations focus on detecting these diseases once they are clinically established [33]. There is currently limited evidence to support their use for predictive purposes during the preclinical or asymptomatic phases of disease development [34].

At this stage, salivary biomarkers are mainly applied in diagnostics and disease monitoring, where they assist in confirming clinical suspicions or assessing disease progression. Their predictive value, however, has yet to be firmly established [35].

The relationship between the oral microbiome and disease remains bidirectional and context dependent. For instance, in autoimmune diseases such as Sjögren's syndrome and systemic lupus erythematosus (SLE), studies suggest that oral microbiome alterations may result more from oral dryness (xerostomia) than from the autoimmune condition itself [36]. Furthermore, van der Meulen et al. [37] reinforces that microbial changes may be secondary to the clinical symptoms rather than a primary etiologic factor.

In the case of periodontitis, dysbiosis microbial environment is a hallmark of disease progression [38]. However, emerging evidence indicates the possibility of a reverse relationship—that an already imbalanced microbiome may contribute to or exacerbate disease susceptibility and severity

This reinforces the idea that microbial shifts can both reflect and contribute to pathology, depending on host factors, environmental influences, and disease stage. Further studies, particularly those involving longitudinal sampling and mechanistic analysis, are needed to unravel whether the microbiome is a trigger, a result, or both in various disease processes.

To transition salivary biomarkers into effective predictive tools, future research must involve well-designed longitudinal and prospective studies. These should aim to correlate biomarker presence or fluctuations with disease onset prior to the appearance of clinical signs. Only through such evidence can saliva-based diagnostics evolve into reliable preventive instruments in precision and personalized medicine.

2.3. Saliva Collection

Because of its molecular composition including several dietary pollutants and the lack of evidence linking salivary and blood indicators, saliva has historically been ignored. A number of recent studies, however, have shown that saliva correlates well with blood indicators, such as glucose and cortisol, suggesting a potential alternative to invasive diagnostics [39–41]. There is a strong relationship between saliva and blood for several markers of inflammation, including IL-6 [42].

Despite previous studies outlining the processes for saliva collection and preservation, it is critical to place these approaches in the context of inflammatory indicators and to outline best practices for accurate collection, handling, and biobanking [43–45]. Even if saliva is being looked at more and more as a possible disease target sample, it would be prudent to compare it to the existing diagnostic methods used to assess inflammatory markers in blood [46].

The most effective way to collect saliva is the "passive drool" technique, which involves gathering saliva in the mouth and then allowing it to flow through designated straws for a certain duration [47]. Anybody, including those without a medical background, can take a saliva sample. Wearable modules make it easy to automate saliva collection, and there are several established techniques for oral sample [48]. Spewing, chewing, and swab-based processes are among the other modalities of collection [49,50].

One less preferable method is chewing, which typically requires the use of wax and permits the collection of stimulated saliva mostly from the parotid glands, as opposed to spitting, which only involves certain salivary glands like the submandibular and minor ones [51]. Regarding the other inflammatory indicators, unstimulated saliva is still the ideal fluid, but the latter is now the suggested medium for measuring CRP concentration in saliva [52].

The last point is that swab-based approaches have been widely employed in COVID-19 tests, and several studies have shown that this approach works especially well when analyzing saliva from youngsters [53,54]. There is evidence that using cotton-based swabs in immunoassay testing causes a large variation in salivary marker concentrations [55]. This method proved effective for viral RNA detection, offering a non-invasive and convenient alternative to nasopharyngeal swabs. The cotton roll technique facilitates passive drool collection, reducing the risk of sample contamination while ensuring an adequate volume for molecular analysis [53]. Given its successful application in infectious disease monitoring, this method may also hold potential for future salivary biomarker research, particularly in detecting systemic diseases and viral infections with minimal patient discomfort [53–55].

While whole saliva is most commonly used due to its ease of collection and non-invasive nature, glandular saliva—collected selectively from the parotid, submandibular, or sublingual glands—can offer greater specificity for biomarker analysis [12]. It allows for the detection of biomarkers secreted predominantly by individual glands, which may be diluted or masked in whole saliva.

Additionally, pre-analytical conditions can greatly impact the composition of saliva and the concentration of biomarkers. Factors such as the time of day, as well as whether the patient has eaten, drunk fluids, smoked, or brushed their teeth, should be standardized to reduce variability [13]. For instance, salivary flow and certain analytes may fluctuate with circadian rhythms or be influenced by recent oral activity. As recommended by Dawes et al. [56], saliva collection protocols should include clear instructions on fasting state and time of collection to improve reproducibility and accuracy in biomarker research.

The accuracy and reliability of salivary biomarkers can be greatly affected by the techniques used to collect the saliva, but no universal strategy has been developed to address this issue [57]. Before conducting any test on saliva, it is important to study how the collection equipment and process affects the recovery of analytes [58]. Using the same approach for all participants included in the same study and throughout all samples obtained for each subject are required after establishing the appropriate collection protocol [59].

Procedures for collecting and storing samples are essential when dealing with real samples. Oral fluid sampling, as opposed to blood or urine sampling, offers a non-invasive, easily accessible, and transportable alternative [60]. Patients with blood coagulation ab-

normalities, newborns, hemophiliacs, and other conditions that can make blood sample collection difficult will find this very helpful [61]. Saliva also makes it easier to track patients' health status and treatment results, as patients are more likely to use it when they need to be monitored on a regular or clinical basis [62]. There is less chance of cross-contamination and employee exposure when using saliva samples; however, there is still a problem with standardizing the collection and storage of saliva samples [63].

It is important to treat or keep saliva samples appropriately after collection. While certain components have extremely short half-lives in saliva, others are highly stable. At room temperature, inorganic compounds tend to remain mostly unchanged [64].

When kept at room temperature, the calcium and magnesium contents of the samples exhibited good stability for at least one week, according to Czégény et al. [65]. In addition, it was shown that calcium and phosphate remained stable for up to two months when kept at −20 °C [65]. In contrast, samples left at room temperature showed a monthly decline of around 10% in cortisol (a steroid hormone) levels [65]. As long as it remains at 5 °C for three months and at −20 °C and −80 °C for one year, cortisol remains stable in saliva. Within 30 min of being collected at room temperature, proteins likewise degraded quickly [65]. To slow deterioration, keep samples at 4 °C and employ protease inhibitors if necessary. The pre-analytical treatment of saliva samples before storage is an alternate method for preserving target analytes. One way to prevent bacterial protease activity from breaking down certain proteins in saliva is to snap-freeze it with glycerol in liquid nitrogen.

In many diagnostic applications, unstimulated saliva is preferred due to its closer reflection of basal salivary composition and its relevance to resting physiological conditions. However, in patients with xerostomia (dry mouth) or other forms of salivary gland dysfunction, collecting sufficient volumes of unstimulated saliva may be difficult or unreliable [59].

In such cases, stimulated saliva, obtained through mechanical or gustatory stimulation (e.g., chewing paraffin wax or applying citric acid), offers a practical alternative. Stimulated saliva provides higher flow rates, reduces collection time, and can help assess the functional reserve of the salivary glands. Moreover, in dry mouth patients, stimulated saliva may better reflect the gland's residual secretory capacity and provide more consistent biomarker detection [62].

That said, stimulated saliva may differ in pH, protein concentration, and electrolyte content compared to unstimulated saliva, which must be considered during data interpretation [63]. Therefore, the choice between stimulated and unstimulated saliva should be disease-specific and protocol-driven, ensuring reproducibility and diagnostic accuracy.

The duration of saliva collection has a significant impact on sample volume, composition, and reproducibility. As noted by Dawes, flow rate and biomarker concentration can vary substantially within the first few minutes of collection [66]. To ensure representative sampling and stable flow rates, it is widely recommended that a minimum collection period of 5 min be used, particularly for unstimulated whole saliva [66].

Shorter collection times may result in insufficient sample volume and increase variability, especially in individuals with reduced salivary flow. A 5 min duration allows for better averaging of fluctuations and ensures the sample reflects a more accurate metabolic and biochemical profile. This standardization is especially important when comparing results across studies or between patient groups.

In the meantime, the source of stimulated saliva depends on the type of stimulation applied, as different salivary glands respond differently [67]. Gustatory stimulation (e.g., using citric acid) tends to increase secretion primarily from the submandibular and sublingual glands, while mechanical stimulation (such as chewing paraffin wax or gum)

predominantly activates the parotid glands [67]. Therefore, the composition and glandular origin of stimulated saliva vary according to the method used.

This distinction is important in salivary biomarker research, as each gland produces saliva with unique biochemical profiles (including enzymes, proteins, and electrolytes), which can significantly influence analytical outcomes. Thus, the choice of stimulation technique should be standardized and aligned with the objectives of the investigation.

2.4. Approaches for the Detection of Salivary Biomarkers

The appropriateness of an approach to biomarker discovery and profiling relies on how the method differs from others in terms of working principle, sample preparation, and interpretation of data [68].

The diagnostic goal, whether for disease detection, monitoring, or prognostic evaluation, dictates which biomarkers are most appropriate. For example, acute inflammatory conditions may require sampling biomarkers, such as interleukins or CRP, which fluctuate quickly and are influenced by circadian rhythms and transient stimuli. In contrast, chronic disease monitoring may focus on more stable markers like cortisol, immunoglobulins, or specific enzymes [69].

Similarly, the collection duration may vary depending on the biomarker concentration and the volume of saliva required. Highly concentrated markers may be detectable in short collections (e.g., 1–2 min), while low-abundance biomarkers may necessitate longer periods (e.g., 5–10 min) to ensure adequate sample volume and detection sensitivity [44].

Furthermore, salivary diagnostics aimed at real-time or point-of-care applications may prioritize rapid, minimal-volume protocols, whereas research or biomarker discovery settings might demand standardized, longer collection procedures to ensure reproducibility and depth of analysis.

Biomarkers and metabolites can be extracted from saliva using a variety of approaches, such as immunological methods, separation methods, and, more recently, electrochemical methods [70], as can be seen in Figure 2:

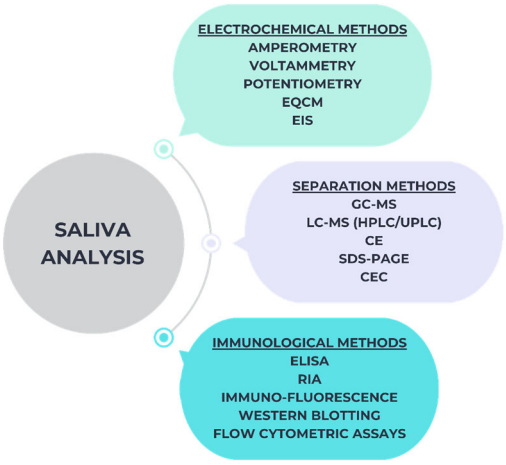


Figure 2. Methods for detection of biomarkers.

2.4.1. ELISAs Method

Enzyme-linked immunosorbent assays (ELISAs) are one of the most common types of immunoassays; they use plates that have been functionalized with antibodies to detect target antigens [71]. They involve the binding of the present antigens in saliva to specific antibodies coated on a microplate. A secondary antibody conjugated with an enzyme is then added, followed by a substrate that reacts with the enzyme to produce a measurable color change. The intensity of the color is directly proportional to the concentration of

the biomarker [72,73]. This method is often considered the gold standard in many tests. Inflammatory indicators like cytokines are among the many targets that ELISA kits can detect, and these kits may be found in both commercial and centralized laboratories. Screening a panel of biomarkers at once utilizing multiplexed technologies allows for more thorough findings with shorter turnaround times by evaluating their concentrations all at once [73].

As strengths for ELISA, we can notice high sensitivity and specificity, well-established in clinical diagnostics, widely available, and cost-effective for single biomarker detection.

Nevertheless, there are limitations to ELISAs that make it impractical to detect numerous biomarkers at once; this is because the ELISA is typically designed to assess a single biomarker [74,75]. Moreover, this test has limited multiplexing capability and is labor-intensive, time-consuming, and prone to interference from sample contaminants. The stability of the reagents, the consistency of the solid phase, the plate washer, the handling of the samples before analysis, the circumstances of kit storage, and the choice and calibration of the pipettes for different volumes are other aspects that might affect the effectiveness of an ELISA test technique [74].

2.4.2. Western-Blotting Method

Another method of identification is immunoblotting performed in the form of Western blotting. It involves the separation of salivary proteins through gel electrophoresis, followed by their transfer onto a membrane. Specific antibodies are then used to target the biomarker of interest [76]. These antibodies are linked to a detection system, such as an enzyme or fluorescent tag, allowing visualization through chemiluminescence or fluorescence.

The pros for Western-blotting method are it is highly specific, useful for protein identification and quantification, and allows for the detection of post-translational modifications, and the limitations are that it is time-consuming, requires significant sample preparation, and is not ideal for large-scale screening.

2.4.3. ICG Method

A third method for biomarkers identification is immunochromatography (ICG), also known as the rapid test or lateral flow assay [77]. This method is fast and easy to use and is often applied in rapid diagnostic tests, such as tests for antibodies or antigens. It works on principle of capillary migration of the saliva sample on a porous strip, where the target biomarkers bind to antibodies labeled with colored or fluorescent particles. The results are visible as colored lines on the test strip [77]. Immunochromatography is valued for its speed, simplicity, and portability and is used for the rapid diagnosis of infections and monitoring of certain systemic conditions [77,78].

A correlation between the signal strength and the antigen abundance on the membrane should be maintained in all immunological procedures.

The strengths for ICG are represented by rapid results, easy to use, portable, and do not require sophisticated laboratory equipment and the limitations by lower sensitivity compared to ELISA, semi-quantitative results, and limited multiplexing capability.

2.4.4. NMR Method

Nuclear magnetic resonance (NMR) is another popular method in metabolomics for the identification of biomarkers in saliva samples [79]. Compared to alternatives, this method has several benefits, including being robust and repeatable. For example, it is able to identify metabolites with a high volatility level without derivatizing the chemical. The sensitivity is weaker compared to linked methods like GC-MS (gas chromatography–mass spectrometry) and LC-MS (liquid chromatography–mass spectrometry), which can identify biomarkers below the detection limit of NMR [80], but sample preparation is less labor-

intensive. Since saliva is a complex matrix, it is necessary to filter and/or centrifuge the spit samples before treatment [79].

NMR is a non-destructive method, provides structural and quantitative information, and requires minimal sample preparation. On the other hand, its cost is high, with low sensitivity for detecting low-concentration biomarkers and complex data interpretation.

2.4.5. CE-MS Method

Another method that has lately gained popularity for identifying biomarkers in saliva is capillary electrophoresis–mass spectrometry (CE-MS) [81]. This approach combines the sensitivity of MS with the electrophoresis-based separation of chemicals according to their electrophoretic mobility as a function of applied voltage, creating a potent and appealing system [82]. Consequently, its use has skyrocketed, with over 50 studies covering metabolite profiling published between 2018 and 2020 [83].

Enzymes consume substrates and produce new molecules more rapidly in chemical processes [84]. All enzymatic tests depend on measuring how much substrate is consumed or what by-product is produced within a specific time frame [84]. Numerous enzymatic approaches to biomarker measurement are already in development [85–88]. One reason these approaches have been so popular is how little they require in terms of resources, tools, and personnel to execute. On the other hand, derivatization and other procedures are typically needed to identify biomarkers at extremely low concentrations, which makes the process more complicated [89].

Distinguished by its high resolution and efficiency in small molecule separation, CE-MS is well-suited for metabolomic studies but requires expensive equipment and a sophisticated analytical setup.

2.4.6. Fluorescence or Chemiluminescence Based Methods

Thanks to their significantly higher sensitivity, other approaches, called separation techniques, including those based on fluorescence or chemiluminescence, are able to circumvent the aforementioned drawbacks [90,91]. In these techniques, several compounds are utilized, some of which can absorb light and emit it at a certain wavelength, while others may do it chemically or independently. The result is that these procedures are far more sensitive than spectrophotometric testing; nevertheless, it is important to note that these approaches are also more expensive because they need specialized equipment.

The advantages and disadvantages of all the above-mentioned techniques are summarized in Table 1 below:

Table 1. Summary on detection of salivary biomarkers.

Technique	Sensitivity	Specificity	Cost	Time Efficiency	Equipment Requirement	Clinical Applicability
ELISA	High	High	Moderate	Moderate	Moderate	Routine clinical use, protein biomarkers
Western blotting	High	Very High	High	Low	High	Protein identification and quantification
Immunochromatography (ICG)	Moderate	Moderate	Low	High	Low	Rapid, portable diagnostics
Nuclear Magnetic Resonance (NMR)	Moderate	High	Very High	Low	Very High	Structural and quantitative biomarker analysis
Capillary Electrophoresis-Mass Spectrometry (CE-MS)	Very High	Very High	Very High	Low	High	Small molecule separation, metabolomics
Smart Biosensors and AI-Integrated Detection	Very High	High	High	Very High	Moderate	Emerging real-time diagnostics, home-use potential

Advanced methods exist, such as microscale thermophoresis, which combines fluorometry's accuracy with thermophoresis's sensitivity and adaptability to provide a quick, strong, and adaptable platform [92]. One notable feature is that it can analyze numerous substrates at once and needs sample sizes less than 10 μL [93]. High equipment and operat-

ing expenses make it difficult to execute these experiments on a broad scale, especially in poorly equipped laboratories, despite their great advantages.

2.5. Smart Biosensors and Intelligent Devices

Because it contains mucopolysaccharides and mucoproteins, saliva has a thick consistency. Analytical results may be off since it is difficult to precisely determine the volume of thick saliva samples [94]. Saliva contains the most possible biomarkers but only at trace amounts. The concentration recorded could be drastically off if the volume is off. Assays need to be very selective and specific, with a low limit of detection, because the concentrations are so tiny [93]. The components of saliva can vary depending on the location in the mouth where the sample is taken and whether it is stimulated or not [95].

The levels of the biomarkers of interest can be affected by oral disorders like gingivitis, changes in pH, and other possible interferences, including smoking, fasting, and hydration level [96].

Different people exhibit rather considerable differences in metabolite concentrations. The fact that many components of saliva have several activities, or even overlapping ones, is probably to blame for the large amount of diversity observed across individuals [97]. Enzymes such as amylases, cystatins, histatins, mucins, and peroxidases work together to fight germs in the mouth [98]. There may also be intra-individual variability to account for, such as seasonal (temperature) changes, diurnal variation, and others. It follows that a single salivary assay may not be accurate. It would be preferable to collect many samples of saliva and do follow-up testing [99].

In order to overcome all these challenges over the past few decades, a plethora of biosensors designed to detect and track salivary biomarkers has been rapidly emerging. When it comes to nanomedicine and technology, biosensors are a thrilling new development [100]. Through its use, healthcare practitioners and people alike may track the development of diseases and the effectiveness of treatments. With the advent of biosensors, people now have a less expensive alternative to doctor's checkups, which means less pressure to pay exorbitant sums of money and better overall health. They are called "point-of-care" devices [101]. This implies they are highly important for early detection and data surveillance, which means patients may receive therapies sooner, and the disease does not worsen.

It is possible to establish a direct or indirect relationship between the biomarker concentration and the electrical signal produced by a biosensor using software. These sensors can employ optical, electrochemical, or piezoelectric transducers to achieve this conversion [102].

These technologies have demonstrated their ability to accurately and rapidly detect biomarkers in saliva. As an example, using a prototype created in 2010 by Yamaguchi and colleagues, salivary cortisol concentrations ranging from 1 to 10 ng mL^{-1} may be accurately measured in under 25 min [103]. To further incorporate these biosensors into medical devices for continuous biomarker monitoring, further research has looked at applications such as mouthguards [104]. An example of this kind of prototype was described by Kim et al., who created a mouthguard that measures uric acid levels in saliva within the normal limits for both healthy and hyperuricemic individuals [105]. Unfortunately, the majority of these studies only conducted laboratory tests, so they still have a way to go before they can be considered clinically and practically applicable to everyday saliva testing.

The device has the potential for additional use in saliva determination, and with the fast development of nanotechnologies over the last 20 years, it is worth thinking about how to enhance its novel features, such as its large surface area, number of active sites, high catalytic efficiency, and biocompatibility of nanomaterial [106]. These advantages

allow the built-in sensor to be more sensitive, respond faster, be smaller, have greater biomolecule stability, and be cheaper. For instance, they hold promises for the quick clinical identification of trace concentrations of salivary biomarkers [107] by allowing for the accurate detection of trace chemicals. Table 2 below encompasses a summary of biosensors found in the literature, with their main principle of action and benefits for patients.

Table 2. Summary on oral biosensors.

Biosensors	Main Action	Benefits
Biosensor for cancer detection [108]	Detects the cancer biomarker CYFRA-21–1 in saliva	Can diagnose cancer at an early stage without invasive surgery or expensive therapies—ELISA is used for identifying purposes.
Alpha Amylase Biosensor [109]	Salivary alpha amylase level detection	Gives information about an individual's diet Gives information on the acidity levels in the mouth cavity as a result of digesting complex carbs
Titanium biosensor [110]	Assessing periodontal health and identifying hazardous amounts of streptococcus Gordonii	Titanium has longevity, resistance to corrosion, and excellent quality The ability to employ CHX as a protective oral health aid expands its application
Urea Smartphone Biosensor [111]	Measuring urea levels from saliva by linking a transducer to a mobile app that calculates an array of urea levels	Real, tangible thing to experiment with and manipulate

An increase in operational efficiency and practicality necessitates biosensors that are more integrated and useful. Utilizing printing technology, microfluidics, organic thin film transistors (OTFTs), paper-based sensing, etc., which are more suitable with smart and flexible devices, is performed with the aim of enhancing the intelligent process of sensors [112–114]. Using these technologies, biosensors for salivary detection may be equipped with sophisticated features, including auto calibration, wireless connectivity, and a microprocessor, in addition to being able to be integrated with other devices [113].

Ensuring the electrodes are well-designed and manufactured is crucial for an intelligent and integrated sensing device. One positive aspect is that it has the potential to enhance the sensing performance of sensitive materials by altering the conventional sensing mode and impacting the functionalities of altered materials [115]. Alternatively, because the overall system's sensing parts are significantly smaller, more room would be available for the design of external integrated devices. Printed sensing electrodes, microfluidics chips, organic thin-film transistors, paper strips, and other self-designed electrodes have found extensive use in salivary biomarker detection recently, towards the goal of realizing the miniaturization, portability, and flexibility of biosensors [116–118], as illustrated in Figure 3:

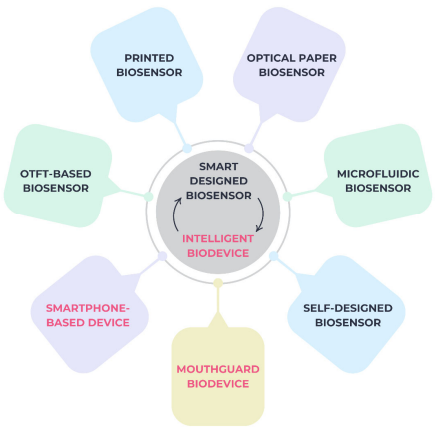


Figure 3. AI technology and biosensor for noninvasive detection of salivary biomarkers.

These electrodes point to a potential new direction for the construction of AI devices in the future. Thus, the development of smart sensors that utilize saliva would be a game-changer in many areas of personal healthcare, including point-of-care (POC) testing, health monitoring, early on-site disease diagnosis, cost-effectiveness, ease of operation, and lack of invasiveness [119].

Artificial intelligence (AI) has increasingly been integrated into salivary biomarker research to enhance diagnostic accuracy and automate biomarker identification [120]. Machine learning models, such as Support Vector Machines (SVMs), Convolutional Neural Networks (CNNs), and Random Forest Classifiers, have demonstrated significant potential in analyzing complex biomarker datasets with high precision [121].

Support Vector Machines (SVMs)—SVMs have been widely used in pattern recognition and classification of biomarker data. They effectively differentiate between disease states based on salivary protein expression profiles, offering an improvement in diagnostic sensitivity compared to conventional statistical approaches [121].

Convolutional Neural Networks (CNNs)—CNNs, primarily used in image and spectral analysis, have been applied to salivary proteomics and mass spectrometry data, enabling automated feature extraction and classification. This has led to more efficient identification of disease-related salivary biomarkers with higher accuracy and lower false-positive rates [122].

Random Forest Classifiers—This ensemble learning method is particularly useful for handling complex, high-dimensional biomarker datasets. Random Forest algorithms have been utilized to predict disease presence by analyzing patterns in salivary metabolomics and proteomics data, often outperforming traditional regression models [123].

Unlike traditional statistical techniques, AI can handle nonlinear relationships, large datasets, and multi-variable interactions, making it a promising tool in personalized medicine and early disease detection using saliva-based diagnostics [123,124].

Future advancements in AI and deep learning will likely refine salivary biomarker analysis further, allowing for real-time, automated diagnostics and precision medicine applications. Integrating AI with biosensors and microfluidic systems could enhance point-of-care diagnostic capabilities, making saliva-based testing more accessible and reliable [125,126].

3. Current Trends and Future Perspectives

The field of salivary biomarkers identification is rapidly evolving, driven by advancements in technology, increased understanding of oral microbiome, and growing interest in non-invasive diagnostic methods. Currently, researchers are focusing on refining existing technologies, developing new biosensors and integrating artificial intelligence for more precise and efficient biomarker detection.

Artificial intelligence and big data analytics are transforming salivary biomarkers research [127]. AI-driven algorithms can process vast amounts of biological data, identifying biomarker patterns and improve diagnostic accuracy [128]. By leveraging machine learning models, researchers can classify disease state, predict progression and develop personalized treatment strategies based on salivary biomarker profile. This approach not only enhances diagnostic capabilities but also reduces human errors and speed up result interpretation [129].

A key challenge in salivary biomarker research remains method standardization. Despite technological advancements, variability in sample collection, processing, and analysis methods affect reproducibility and cross-study comparisons [130]. Standardized protocols, including unified sample storage conditions and validated biomarker panels, are essential to ensure consistency and reliability in clinical applications. Efforts are being made

to establish globally accepted guidelines to facilitate the integration of salivary diagnostics into routine clinical practice.

There has also been recent interest in developing portable diagnostic tools for the non-invasive detection of illness. Their low cost, quick turnaround time, and ease of use make them stand out when compared to more traditional laboratory procedures [131]. For these reasons, they are a great substitute for traditional laboratories in inaccessible or faraway locations where it would be too costly to establish one up [132]. Moreover, they may be effortlessly carried and used in mobile diagnostic sites due to their portability and low energy consumption requirements [30,102,103].

Despite these advancements, several challenges remain. One of the key issues is ensuring the reproducibility and reliability of salivary biomarker findings across different populations. Variability in salivary composition due to external factors such as diet, hydration, and circadian rhythm can impact biomarker stability, complicating standardization efforts. Future research must address these inconsistencies by establishing robust normalization techniques and universal testing protocols.

Moreover, regulatory and ethical considerations surrounding salivary diagnostics must be carefully considered. As these technologies become more integrated into everyday healthcare, questions regarding patients' privacy, data security, and regulatory compliance will need to be addressed to ensure safe and effective implementation [125].

Looking toward the future, interdisciplinary collaboration will play a crucial role in advancing salivary biomarker research. The convergence of biotechnology, nanomedicine, bioinformatics, and clinical research is expected to accelerate the translation of laboratory findings into real-world applications. Future studies will likely focus on expanding biomarker databases, validating multi-biomarker panels, and refining analytical techniques to enhance specificity and sensitivity.

4. Conclusions

Saliva-based biomarker identification is an innovative, non-invasive alternative to traditional diagnostic methods, offering a painless and convenient approach for early diagnostic disease and health monitoring.

The integration of advanced techniques, such as nanotechnology, artificial intelligence, and biosensors, has significantly improved the sensitivity and specificity of biomarker detection, enabling precise and real-time diagnostics.

Despite technological progress, the lack of standardization protocols for saliva collection, storage, and biomarker validation remains a significant barrier to widespread clinical adoption.

The current trends in salivary biomarker identification reflect a shift toward more sophisticated, non-invasive, and technology-driven approaches. With ongoing advancements in biosensor technology, AI integration, and standardization efforts, salivary diagnostics are poised to become a cornerstone of modern precision medicine, offering accessible and reliable tools for early disease detection and personalized healthcare.

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Abbreviations

The following abbreviations are used in this manuscript:

CVD	Cardiovascular Disease
RNA	Ribonucleic Acid
DNA	Deoxyribonucleic Acid
AI	Artificial Intelligence
mRNA	Messenger RNA
TNF-α	Tumor Necrosis Factor-alpha.
IL-6	Interleukin 6
MMP_s	Matrix Metalloproteinases
miRNA	MicroRNA
MDA	Malonedialdehyde
CRP	Protein C Reactive
ELISA	Enzyme Linked Immunosorbent Assays
ICG	Immunochromatography
NMR	Nuclear Magnetic Resonance
GC-MS	Gas Chromatography-Mass Spectrometry
LC-MS	Liquid Chromatography-Mass Spectrometry
CE-MS	Capillary Electrophoresis-Mass Spectrometry
MS	Mass Spectrometry
µL	Microliter
OTFTs	Organic Thin Film Transistors
POC	Point-of-Care

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