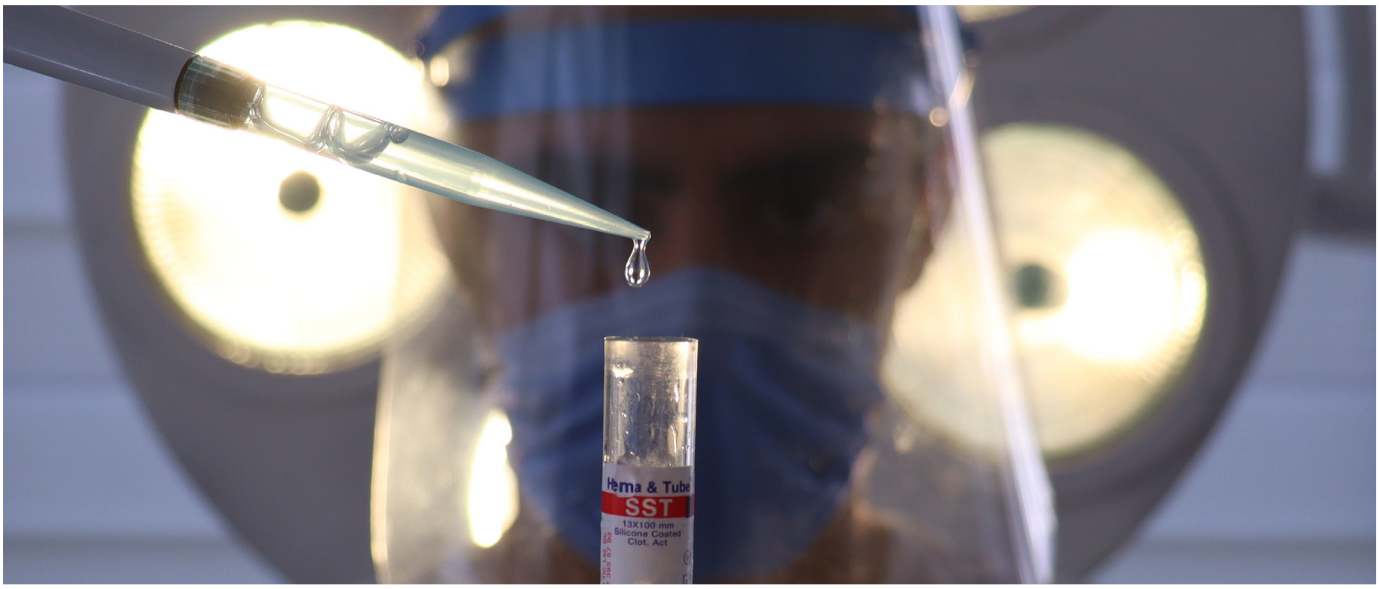
Review

What is the evidence that saliva is a suitable alternative to serum in the diagnosis of systemic disease? A review of the literature

Liam Fletcher
Year 4, Dentistry, University of Bristol.
Email: oj18783@bristol.ac.uk



Abstract

The need for rapid, reliable diagnostic methods has never been more evident in the light of the COVID-19 pandemic. Salivary diagnostics are a non-invasive, quick and inexpensive tool in the detection of disease and their potential are yet to be fully harnessed. The aim of this review article is to assess the feasibility of using saliva as an alternative biological fluid to serum in the diagnosis of systemic disease. The objectives of this article were to: (1) identify if the physiological properties of saliva support its use in being able to reflect various physiological states of the body; (2) evaluate the current evidence with regard to the use of salivary biomarkers compared to conventional serum biomarkers in the detection of some of the most prevalent systemic diseases; and (3) establish whether current technology potentiates the clinical application of salivary diagnostics.

Abbreviations

AMI - Acute myocardial infarction
CTnI - Cardiac troponin I
ECG – Electrocardiogram
ELISA - Enzyme-linked immunosorbent assay
GCF - Gingival crevicular fluid
HIV – Human immunodeficiency virus
LFT - Lateral flow test
LOC - lab-on-chip
PCR – Polymerase chain reaction
POC - Point-of-care

Introduction

A diagnostic biomarker is a biological measurement that can be used to confirm the presence or absence of a disease and contributes significantly to modern-day diagnosis. Perhaps the most consequential benefit of diagnostic biomarkers is their ability to detect disease in the absence of physical signs and symptoms, removing the need to rely on these parameters alone. This, in turn, facilitates earlier diagnosis and, thus, earlier prevention or treatment, most importantly in diseases that exhibit late-stage presentation. In addition, diagnostic biomarkers have the potential to expedite clinical trials when used as a surrogate endpoint and pave the way to redefine the classification of a disease using biological measurements as opposed to physical characteristics.[1] The conventional method to identify diagnostic biomarkers in the body is via blood serum analysis, yet this requires an invasive method of collection and extensive training from the practitioner. Saliva has an array of biological functions and cannot be overlooked as a non-invasive, quick and inexpensive alternative diagnostic fluid[2,3] as it possesses an abundance of informative molecules for diagnosis, including DNA, RNA and proteins.[4] This review will investigate the evidence as to whether saliva can be utilised as an alternative to serum as a diagnostic tool for systemic disease by: (1) considering the properties influencing its diagnostic potential; (2) summarising our understanding of salivary biomarkers versus conventional serum biomarkers in three prevalent systemic diseases; and (3) discussing the feasibility of using saliva with current diagnostic instrumentation in a clinical setting.

Properties of saliva influencing its diagnostic potential

Saliva arises from a variety of sources including the major salivary glands and minor salivary glands. The extent of contribution of saliva from these sources varies depending on whether the saliva is stimulated or unstimulated (see **Table 1**).[5] Saliva production relies on a series of active and passive diffusion mechanisms (**Figure 1a**), which lead to a final product rich in protein, electrolytes and more (see **Table 1**).[6]

In the mouth, saliva is mixed with gingival crevicular fluid (GCF), which is the fluid around the necks of teeth (see **Figure 1b**). GCF contains cytokines, immunoglobulins, host enzymes, serum proteins and inflammatory cells. The contributions from GCF to the saliva occur via capillary leakage and provide dynamic, real-time information pertaining to biomolecules that can be found in serum.[7]

Salivary flow rates exhibit high intra- and interindividual variation due to factors like hydration status, age, disease and medication, with higher flow rates reducing and lower flow rates elevating biomarker concentrations.[8] For example, a 38% reduction in flow rate was observed in the elderly,[9] suggesting that concentrations of salivary biomarkers may be elevated in this cohort. This decline is often attributed to the physiological ageing process; however, polypharmacy and the presence of disease is more often the explanation. Thompson *et al*. established the link between polypharmacy and hyposalivation, with a number of medications having a severe effect on salivation. In a study of elderly people, participants were found to be taking a variety of drugs, including antihypertensives, antidepressants, analgesics, and statins. Hyposalivation was associated with use of antidepressants or bronchodilators.[10] Thus, considering that systemic disease is more prevalent in an ageing demographic, it is important to be aware that associations between salivary biomarker concentration and disease could be heavily affected by the reduction in salivary flow rate and volume variability. Consequently, caution is warranted when interpreting study data with regard to biomarker concentrations associated with disease, most notably with studies that do not account for age differences.

Salivary biomarkers associated with common systemic diseases

Cardiovascular disease Cardiovascular disease is one of the main causes of death globally. The burden of this disease is on the rise, with more than 5 million additional deaths reported in 2015 compared to 1990,[11] highlighting the need for rapid diagnosis to reduce mortality. The most preferable biomarker in serum for the diagnosis of acute myocardial infarction (AMI) is cardiac troponin I (cTnI), a protein released into the blood when the myocardium is damaged. Foley *et al*. reported that salivary cTnI levels exhibit a positive correlation with serum cTnI, yet consistently show lower concentrations.[12] The participants in the study by Foley and colleagues were chosen based on the fact that they were undergoing surgical intervention for heart disease (alcohol septal ablation or percutaneous coronary intervention). Therefore, cardiac damage would be largely influenced by surgical and human factors and, thus, may not accurately mirror the event of an AMI. Nonetheless, Mishra *et al*.,[13] who utilised participants suffering from AMI within 24 hours, reported a statistically significant elevation in salivary cTnI compared to the control, confirming that saliva can reflect cardiac damage. Despite the low concentrations of cTnI reported in both studies, there is evidence to suggest the concentration of this marker in saliva is sufficient for detection.[14] Furthermore, Floriano *et al*. investigated the use of a panel of 3 salivary-based biomarkers, consisting of C-reactive protein, myoglobin and myeloperoxidase, as a screening tool for AMI. This saliva-based biomarker panel was shown to have a sensitivity and specificity similar to serum diagnostics when used in conjunction with an electrocardiogram (ECG).[15] This study provides a good level of evidence according to the Oxford Centre of Evidence-Based Medicine (Level 2b, which is 'an exploratory cohort study with good reference standards').[16] The advent of highly sensitive tests potentiates the use of salivary cTnI in AMI diagnosis, yet the utility of a panel of biomarkers may offer the best diagnostic capabilities.

Diabetes An estimated 451 million adults live with diabetes, and this is projected to rise to 693 million by 2045. Diabetes is a major cause of cardiovascular, kidney and liver disease[17] yet, in the case of type 2 diabetes, is preventable and reversible in its early stages. Therefore, the need for early identification is paramount in facilitating early prevention. A diabetes diagnosis is confirmed by the detection of a fasting blood glucose of greater than 7 mmol/l or a two-hour postprandial plasma glucose concentration greater than 11.1 mmol/l.[18] Unstimulated whole saliva and serum glucose levels are shown to correlate, with higher concentrations being found in diabetic patients. One study reported that when a salivary glucose level is equal to or greater than 0.25 mmol/l, a diabetes diagnosis could be made with 78% sensitivity and 80% specificity.[19,20] Contrary to this, Wang *et al*. reported no correlation between unstimulated whole saliva and serum glucose levels, but did note an association between parotid gland-derived saliva and serum glucose.[21] Parotid gland synthesis of saliva fluctuates considerably, and saliva production is increased by 30% when stimulated vs unstimulated.[4] The variability in the extent to which parotid glad-derived saliva contributes to whole saliva may explain the differing results found regarding the association between saliva glucose and serum glucose, as well as casting doubt on the reliability of using a predetermined glucose value (as used with serum glucose) when using saliva to diagnose diabetes.

The scope of salivary diagnostics with regard to diabetes is yet to be expanded to include a panel of biomarkers, such as that suggested for the diagnosis of cardiovascular disease. Additional salivary biomarkers demonstrating a statistically significant elevation between diabetic and healthy patients include salivary amylase, calcium and phosphorus, all of which are potential candidates for the construction of a biomarker panel, which may provide more consistent and reliable diagnostic results.[19]

Human immunodeficiency virus Around 1 million people die every year because of an underlying infection with human immunodeficiency virus (HIV), with the majority being concentrated in sub-Saharan Africa.[22] Considering the greatest prevalence of HIV is within low-income countries, the availability of simple and cheap diagnostic tests is hugely beneficial. The use of saliva as a diagnostic tool for HIV forms the basis of one of the most successful salivary diagnostic tests, known as OraQuick. This test detect immunoglobulins against HIV in saliva that have passed from serum via oral mucosa transudation.[25] Belete *et al*. and Deville and Tempelman reported sensitivities and specificities of approximately 99% and 100%, respectively, when using OraQuick to diagnose HIV;[23,24] these validating cohort studies present a high level of evidence (Level 1b).[16] However, a study that included participants taking antiretroviral medication showed that the sensitivity of OraQuick is lower in cases with a reduced viral load, whilst blood serum analysis provided a constant sensitivity and specificity of 100% regardless of viral load,[26] maintaining its status as the gold standard for HIV diagnosis. A solution to this could be the utilisation of a panel of biomarkers, such as salivary malondialdehyde (a factor that is positively associated with oxidative stress, which is elevated in HIV-infected patients)[27] in conjunction with immunoglobins.

See **Table 2** for a summary of all biomarkers discussed in this review.

Application of salivary diagnostic instrumentation to clinical practice

Biomarkers can come in a variety of forms, including DNA, RNA and proteins. As such, an array of laboratory tests can be utilised to

Table 1. Sources of saliva. The physical properties and percentage contributions of saliva from its various sources.[5]

Source	Acinar type	Viscosity	Composition	Unstimulated saliva (%)	Change from unstimulated to stimulated saliva (%)[a]
Parotid gland	Serous	Watery	Amylase, proline- rich proteins, agglutinins, and small amounts of cystatins, lysozymes, and extraparotid glycoproteins	20	+30
Submandibular gland	Mixed (predominately serous)	Semi-viscous	All components of serous and mucous secretions, including high levels of cystatins	65	-
Sublingual gland	Mucous	Viscous	Mucin glycoprotein-1, mucin glycoprotein-2, lysozymes	7-8	-
Minor salivary glands	Mucous	Viscous	Mucin glycoprotein-1, mucin glycoprotein-2, lysozymes	10	-
GCF	-	Watery	Electrolytes, inflammatory mediators, cellular components, host enzymes, and metabolic and tissue breakdown products	<7	-

[a]Data for stimulated saliva is only reported for the parotid gland.

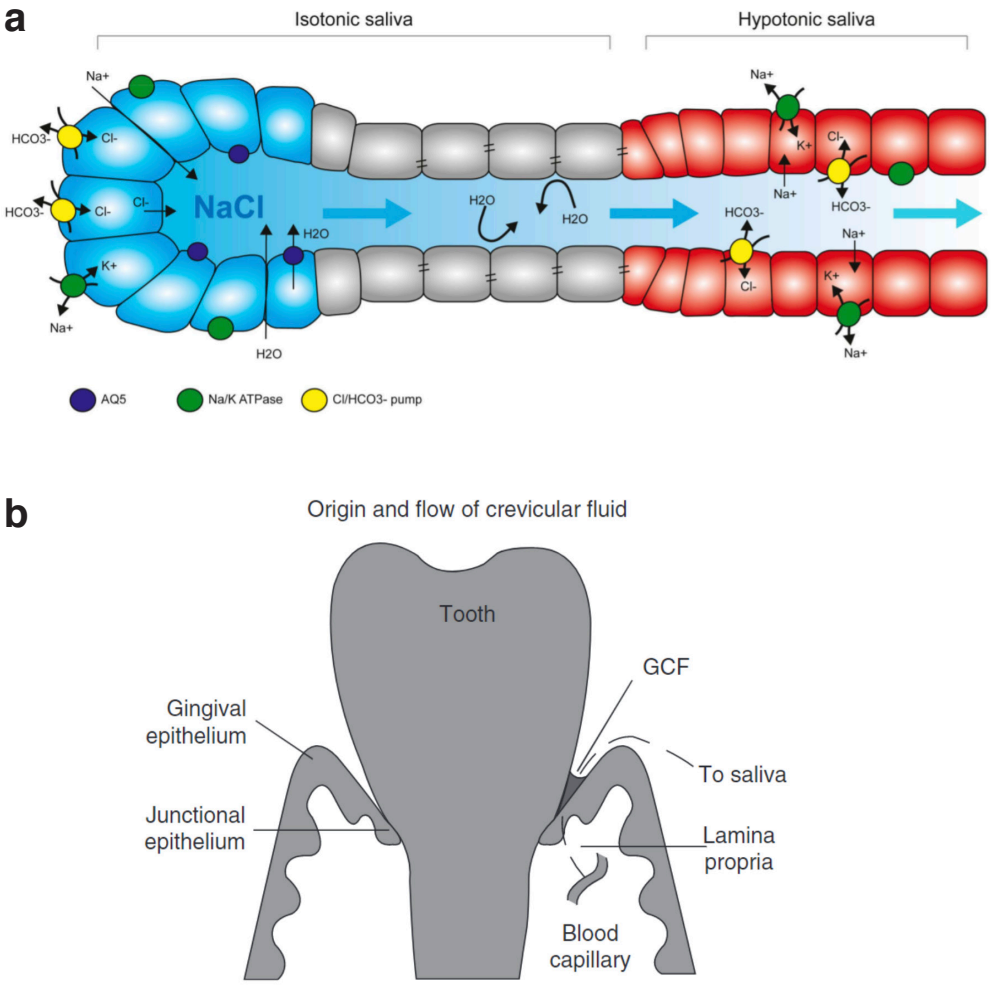


Figure 1. The stages of saliva production. (**a**) Saliva production begins with the formation of a primary isotonic saliva, containing sodium chloride (NaCl), via active and passive diffusion. This is subsequently modified and NaCl is replaced with bicarbonate (HCO_3^-) and potassium (K^+) ions, resulting in a hypotonic product. Figure from Porcheri and Mitsiadis.[7] (**b**) GCF is produced via transudation from the blood capillary, utilising transcellular and paracellular transport mechanisms, followed by passage through the lamina propria connective tissue and, finally, junctional and sulcular epithelium filtration into the gingival sulcus. GCF then passes to saliva. Reprinted from Challacombe *et al.*[6], with permission from Elsevier.

detect biomarkers, being specific for a given type of marker. Some conventional laboratory tests include polymerase chain reaction (PCR) and DNA/RNA sequencing, microarrays for measuring DNA, microRNA or protein analysis, culture techniques for the maintenance or growth of biological samples, and enzyme-linked immunosorbent assay (ELISA). ELISA is one of the most sensitive and omnipresent diagnostic tools in healthcare,[28] which can also be applied in salivary diagnostics. However, the biomolecules in saliva, most notably peptides, are susceptible to rapid degradation and require immediate processing or expensive requisites to preserve the sample in a clinical setting.[29] This renders methods for peptide analysis, such as ELISA, less preferable in clinical care. A range of point-of-care (POC) instruments have been identified with sufficient sensitivities for use in salivary diagnostics, such as the lab-on-chip (LOC) systems.[30] These systems possess one laboratory function or include several functions on a single integrated circuit, facilitating rapid results, thus making them more suitable for clinical application for salivary diagnostics. An example includes the lateral flow tests (LFTs) currently being used to detect the presence of SARS-CoV-19 during the COVID-19 pandemic (**Figure 2**). LFTs can be performed by patients and provide

Table 2. A summary of the salivary biomarkers for cardiovascular disease, diabetes, and HIV discussed in this article.

Salivary biomarker/ biomarker panel	Advantages	Disadvantages	Study authors; level of evidence[a]
Cardiovascular disease			
C-reactive protein, myoglobin and myeloperoxidase	• Excellent sensitivity and specificity (80-90%) when used with ECG	• Needs to be used in conjunction with ECG recordings to achieve optimum specificity and sensitivity	Floriano *et al.*;[15] Level 2b
cTnI	• Direct correlation with serum troponin levels • Established laboratory tests already in use for serum diagnostics	• Significantly lower concentrations compared to serum, making detection difficult • Tests with greater sensitivities required for confident diagnosis	Foley *et al.*;[12] Level 3b Mishra *et al.*;[13] Level 3b
Diabetes			
Glucose	• Potential correlation with serum glucose levels • Method of detection quick and easy	• Results heavily influenced by extent of saliva contribution by various glands • Glucose concentrations considerably lower when compared to serum	Ladgotra *et al.*;[19] Level 3b Mrag *et al.*;[20] Level 2b Wang *et al.*;[21] Level 3b
Amylase	• Significant elevation in salivary concentrations in diabetic patients compared to people without diabetes • Greater concentrations in saliva relative to serum	• Poor stability when not kept under optimum conditions due to enzymatic properties • Requires rapid processing	Ladgotra *et al.*;[19] Level 3b Mrag *et al.*;[20] Level 2b
HIV			
HIV-1/2 antibody	• Excellent sensitivity and specificity (99-100%) • Rapid testing kits already available (e.g. OraQuick)	• Viral load heavily influences sensitivity of salivary diagnostics, unlike serum diagnostics	Belete *et al*;[23] Level 1b Deville and Tempelman;[24] Level 1b
Malondialdehyde	• Accurately reflects oxidative stress in HIV-positive patients • Can be used in conjunction with antibody testing to strengthen diagnostic capabilities	• Insufficient evidence to be used alone	Amjad *et al.*;[27] Level 3b

[a]Level of evidence according to the Oxford Centre of Evidence-Based Medicine:[16] Level 1b, validating cohort study with good reference standards; Level 2b, exploratory cohort study with good reference standards; Level 3b, non-consecutive study or without consistently applied reference standards.

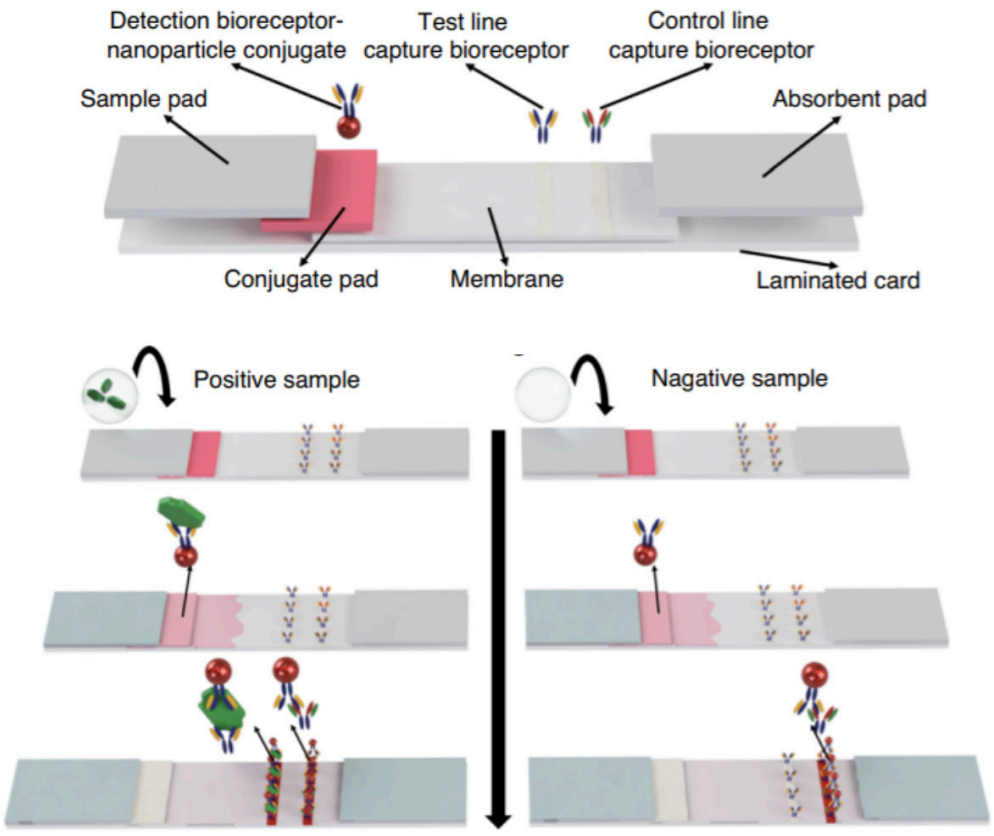


Figure 2. The mechanism of a LFT used to detect the presence of SARS-CoV-19 during the COVID-19 pandemic. The sample is placed onto the sample pad and works its way along the strip by the capillary action stimulated by the absorbent pad. Target analytes that are present bind to the immunofluorescent antibody on the conjugate pad. The combined target and antibody will travel along the nitrocellulose membrane and bind to a binding reagent. This produces a distinct fluorescent line on the membrane, with a darker colour corresponding to a greater concentration of the analyte. Adapted from Parolo *et al.*[31] by permission from Springer Nature.

rapid results, within 30 minutes. This removes the need for testing facilities and trained staff, thus reducing the overall expense and time consumption required for laboratory testing. Recent data reported by the Department of Health and Social Care presents an LFT sensitivity of 50.1% and specificity of 99.72-100%, while the sensitivity of PCR is 94.2-100% and its specificity is 100%.[32] Although the sensitivity of the LFT renders it inferior to PCR, its high specificity means that a positive result does not need to be confirmed with further testing, making it a useful screening tool when laboratory resources are scarce. Thus, it is evident that there are rapidly emerging diagnostic tools that pave the way for salivary diagnostics in the clinical settings upon the validation of appropriate biomarkers.

Conclusion

Salivary diagnostics offers less invasive sample collection methods and lower costs of procurement than blood diagnostics by circumventing the need for expensive training and laboratory testing. Modern technology offers unprecedented application of salivary diagnostics in clinical practice, with advents such as rapid LFTs, which have played an instrumental role in the diagnosis of COVID. The literature presents an abundance of possible biomarkers, with biomarker panels having the highest potential, with some tests having sensitivities and specificities comparable to that of blood serum, most notably for HIV diagnosis. It is suggested that the properties of saliva, such as high flow-rate variability, as well as the range of contributions from different saliva sources to the total saliva volume, both between and within patients, may be the main barrier to reaching a consensus on which salivary components may be useful biomarkers for disease. Consequently, it is likely that there are many salivary biomarkers that remain to be elucidated and further investigations should account for properties that may be confounding to the use of saliva for disease diagnosis. In addition, future research should focus on the identification of new salivary biomarkers, as well as those already identified, with large-scale trials and subsequent validation of findings before saliva can be utilised as a reliable alternative to blood serum in the diagnosis of disease.

Acknowledgements With special thanks to Dr Angela Hague (University of Bristol, Bristol, UK) who helped with editing this review.

Contribution statement The author conducted the literature search, drafted the review, and approved the final version for inclusion in Inspire.

References

1. Califf RM. Biomarker definitions and their applications. Experimental Biology and Medicine. 2018;243(3):213-221
2. Roblegg E, Coughran A, Sirjani D. Saliva: An all-rounder of our body. European Journal of Pharmaceutics and Biopharmaceutics. 2019; 142:133-41.
3. Javaid MA, Ahmed AS, Durand R, et al. Saliva as a diagnostic tool for oral and systemic diseases. Journal of Oral Biology & Craniofacial Research. 2016;6(1):66-75.
4. Humphrey SP, Williamson RT. A review of saliva: normal composition, flow, and function. Journal of Prosthetic Dentistry. 2001;85(2):162-9.
5. Iorgulescu G. Saliva between normal and pathological. Important factors in determining systemic and oral health. Journal of Medicine and Life. 2009; 2(3):303-307
6. Challacombe SJ, Shirlaw PJ, Thornhill MH (2015). Immunology of Diseases of the Oral Cavity. In: Mestecky J, Strober W, Russell MW (eds), Mucosal Immunology, 4th edn, Academic Press, London.
7. Porcheri C, Mitsiadis TA. Physiology, Pathology and Regeneration of Salivary Glands. Cells. 2019; 8(9), 976.
8. Gill SK, Price M, Costa RJS. Measurement of saliva flow rate in healthy young humans: influence of collection time and mouthrinse water temperature. European Journal of Oral Sciences. 2016;124(5):447-53.
9. Xu F, Laguna L, Sarkar A. Aging-related changes in quantity and quality of saliva: Where do we stand in our understanding? Journal of Texture Studies. 2019;50(1):27-35.
10. Thomson WM, Ferguson CA, Janssens BE, et al. Xerostomia and polypharmacy among dependant older New Zealanders: a national survey. Age and Ageing. 2021; 50(1):248-251.
11. Roth GA, Johnson C, Abajobir A, et al. Global, Regional and National Burden of Cardiovascular Disease for 10 causes, 1990 to 2015. Journal of the American College of Cardiology. 2017;70(1):1-25.
12. Foley JD, Sneed JD, Steinhubl SR, et al. Oral fluids that detect cardiovascular disease biomarkers. Oral Surgery Oral Medicine Oral Pathology Oral Radiology. 2012;114(2):207-14.
13. Mishra V, Patil R, Khanna V, et al. Evaluation of Salivary Cardiac Troponin-I as Potential Marker for Detection of Acute Myocardial Infarction. Journal of Clinical and Diagnostic Research. 2018;12(7):ZC44-ZC7.
14. Miller CS, Foley JD, Floriano PN, et al. Utility of Salivary Biomarkers for Demonstrating Acute Myocardial Infarction. Journal of Dental Research. 2014;93(7):72-9.
15. Floriano PN, Christodoulides N, Miller CS, et al. Use of Saliva-Based Nano-Biochip Tests for Acute Myocardial Infarction at the Point of Care: A Feasibility Study. Clinical Chemistry. 2009;55(8):1530-8.
16. Centre of Evidence Based Medicine (2009). Oxford Centre of Evidence-Based Medicine: Levels of Evidence. Available from: https://www.cebm.ox.ac.uk/resources/levels-of-evidence/oxford-centre-for-evidence-based-medicine-levels-of-evidence-march-2009. Accessed: 18 July 2021.
17. Lin X, Xu Y, Pan X, et al. Global, regional, and national burden and trend of diabetes in 195 countries and territories: an analysis from 1990 to 2025. Scientific Reports. 2020: 10, 14790.
18. Diabetes UK (2021). Diagnostic Criteria for Diabetes. Available from: https://www.diabetes.org.uk/professionals/position-statements-reports/diagnosis-ongoing-management-monitoring/new_diagnostic_criteria_for_diabetes. Accessed: 27 April 2021.
19. Ladgotra A, Verma P, Raj SS. Estimation of Salivary and Serum Biomarkers in Diabetic and Non-Diabetic Patients - A Comparative Study. Journal of Clinical and Diagnostic Research. 2016;10(6):ZC56-ZC61.
20. Mrag M, Kassab A, Omezzine A, et al. Saliva diagnostic utility in patients with type 2 diabetes: Future standard method. Journal of Medical Biochemistry. 2020;39(2): 140-148.
21. Wang BB, Du J, Zhu Z, et al. Evaluation of Parotid Salivary Glucose Level for Clinical Diagnosis and Monitoring Type 2 Diabetes Mellitus Patients. Biomed Research International. 2017; 2017:2569707.
22. Murray CJL, Kyu HH, Frank TD, et al. Global, regional, and national incidence, prevalence, and mortality of HIV, 1980-2017, and forecasts to 2030, for 195 countries and territories: a systematic analysis for the global burden of disease, injuries, and risk factors study. The Lancet. 2017; 6(12): E831-E859.
23. Belete W, Deressa T, Feleke A, et al. Evaluation of diagnostic performance of non-invasive HIV self-testing kit using oral fluid in Addis Ababa, Ethiopia: A facility-based cross-sectional study. Plos One. 2019;14(1):10.
24. Deville W, Tempelman H. Feasibility and robustness of an oral HIV self-test in a rural community in South-Africa: An observational diagnostic study. Plos One. 2019;14(4):13.
25. Jyoti B, Devi P. Detection of human immunodeficiency virus using oral mucosal transudate by rapid test. Indian Journal of Sexually Transmitted Diseases and AIDS. 2013;34(2):95-101.
26. Jaspard M, Le Moal G, Saberan-Roncato M, et al. Finger-Stick Whole Blood HIV-1/-2 Home-Use Tests Are More Sensitive than Oral Fluid-Based In-Home HIV Tests. Plos One. 2014;9(6):5.
27. Amjad SV, Davoodi P, Goodarzi MT, et al. Salivary Antioxidant and Oxidative Stress Marker Levels in HIV-positive Individuals. Combinatorial Chemistry & High Throughput Screening. 2019;22(1):59-64.
28. Aslam B, Basit M, Nisar MA, et al. Proteomics: Technologies and Their Applications. Journal of Chromatographic Science. 2017;55(2):182-96.
29. Da Silva ACB, Da Silva DR, de Macedo Ferreira SA, et al (2015). Salivary Diagnostics, Current Reality and Future Prospects. In: Virdi MS (eds), Emerging Trends in Oral Health Sciences and Dentistry. IntechOpen, London, pp 673-690.
30. Khan RS, Khurshid Z, Asiri FYI. Advancing Point-of-Care (PoC) Testing Using Human Saliva as Liquid Biopsy. Diagnostics. 2017;7(3):39.
31. Parolo C, Sena-Torralba A, Bergua JF, et al. Tutorial: design and fabrication of nanoparticle- based lateral-flow immunoassays. Nature Protocol. 2020; 15:3788–3816.
32. Department of Health and Social Care (2021). Lateral flow device specificity in phase 4 (post marketing) surveillance. Available from: https://www.gov.uk/government/publications/lateral-flow-device-specificity-in-phase-4-post-marketing-surveillance. Accessed: 18 July 2021.