Review Article

Lite Image Analysis Module Salivary Diagnostic Tool - A Review

ABSTRACT

Background: Saliva is a versatile biofluid that can help in detecting any oral or systemic disease of an individual. Saliva seems to be clinically an informative biofluid for easy prognosis of a disease and clinical or laboratory diagnosis of oral as well as many systemic diseases. It has some specific soluble biological markers that can be considered as an ideal approach for early detection of diseases.

Aim: The aim of this paper was to review the recent developments of the Lite Image Analysis Module.

Conclusion: Thus, this technology primarily helps in a rapid assessment of salivary biomarker levels signifying the probable systemic and oral condition of an individual both in quantitative as well as qualitative manner. Adopting a simple and quick technique, LIAM can be used effortlessly in some rural remote areas as well where advanced high technology laboratories have not yet reached.

Clinical Significance: It is one of the recent advancements in the world of salivary diagnosis LIAM (Lite Image Analysis Module) that is a portable, light, hand-held scanning device which magnificently integrates a distinct analyte identification system that detects the type of analytes being tested and transfer the report straight to a smartphone or a Bluetooth devices.

KEY WORD:

LIAM, Lateral Flow Test, Non-invasive, Oral disease, Point of care salivary diagnostics, Saliva, Super•SAL, VerOFy®

1. INTRODUCTION

Early phase detection of human diseases like cancer, cardiovascular and metabolic disorders turns out to be a challenging situation in a healthcare setting. Most of them require specialised clinical assessment along with some laboratory testing as well. Salivary diagnostic could pave a path as an effective method for the early diagnosis of these diseases.[1] The clinical importance of saliva can have a varied range starting from the forensic field to drug monitoring followed by diagnosis of various local as well as systemic diseases.[2]

Since time immemorial, saliva has been an indicator for evaluating the status of the human body. Back in China, an individual was suspected of being guilty, if he fails to swallow a mouthful of dry rice. It was based on the notion that a person may not be able to form a dry rice bolus and swallow it because of anxiety and probably guilt, since a feeling of anxiety and guilt would decrease the saliva production needed for easy swallowing. Reports gradually flourished that the parotid saliva flow rate relies on different factors such as nervousness, morality, judgement and introversion. [3]

As it is known, saliva reflects the body's health as it comprises hormones, proteins, enzymes which are also often present in standard blood tests to identify any disease. Our salivary glands produce approximately 600 ml/day of saliva that comprises of various organic and inorganic components whose imbalance can lead to a variety of systemic diseases.[4,5] The

Saliva of mouth is made up of salivary gland secretions, gingival crevicular fluid, transudates of mucosa, various microbes, debris of food as well as desquamated cells of epithelia.[6] However, unlike blood, collection of saliva is easy, less painful to patients, less infectious for healthcare provider during handling and approximately most of the analytes that are in blood are also found in saliva.[7,8]

Most of the times it turns out to be a challenge to diagnose any systemic disorder without the use of additional invasive investigations. To tackle these conditions, medical experts are identifying disease biomarkers which are non-invasive and will give a quick diagnosis of any condition. Over the past few years, newly emerging salivary diagnostic tools have shown a remarkable impact in the field of biomedical research and clinical diagnosis.

However, as previously mentioned unlike blood, salivary diagnostics offers a simple, cheap, atraumatic and stress-free procedure for disease detection as biomarkers [9] present in the saliva can be very useful for early detection of disorder to prevent harmful consequences. Salivary diagnostics can be used for various diseases and conditions such as:

1.1 Autoimmune disorder

Sjogren's Syndrome (SS): Characterized by reduced secretion from the salivary glands and lacrimal glands along with endocrine disturbance. Saliva as a diagnostic tool is of great importance in the diagnosis of SS. Salivary protein analysis revealed an increase in lactoferrin, beta 2 microglobulin, lysozyme C and cystatin C levels. However, there is a decrease in the level of salivary amylase and carbonic anhydrase.[10] **Multiple Sclerosis:** An inflammatory disease characterized by loss of myelin and scarring caused due to destruction of myelin producing cells by the immune system. In salivary diagnosis, it shows a reduction in IgA production. [11] **Sarcoidosis:** It is an inflammatory disease of the lymph node, lungs, liver, skin or other tissues. Salivary diagnostics demonstrates a decrease in salivary flow along with a reduction in the enzyme activity of alpha-amylase.[12]

1.2 Dental caries and periodontal diseases: The increased numbers of Streptococcus mutans and lactobacilli in saliva have been associated with an increased caries prevalence and root caries.[13] Periodontal diseases have been associated with increased level of aminotransferase and alkaline phosphatase. In type 2 diabetes mellitus lower levels of pIgR, Arp 3, CA VI, and IL-1Ra were observed whereas higher levels of PLS-2, LEI and IGJ chain were observed.[14]

1.3 Cardiovascular system: Cardiovascular disease is a major cause of death worldwide. Determination of total serum amylase and salivary amylase activity have been made before and after cardiovascular surgery. In a ruptured aortic aneurysm, the salivary alpha amylase level is increased.[15]

1.4 Drug level monitoring: Saliva has gained importance for its use for drug monitoring and detection of illicit drugs. Saliva is used to detect the presence of nicotine, cannabinoids, cocaine, phencyclidine, opioids, barbiturates, diazepines, amphetamines, and ethanol. Only the unbound portion of the drug in serum is available for diffusion into saliva the unbound fraction of a drug is generally the pharmacologically active fraction. This may represent an advantage of drug monitoring in saliva in comparison with drug monitoring in serum, where both bound and unbound fractions of a drug can be detected. Salivary nicotine levels can be used to monitor exposure to tobacco smoke. [16]

1.5 Forensics: Salivary analysis has been widely used for forensics. The salivary samples can be easily obtained from glasses, cigarettes, food products, envelopes, and other sources. DNA

is mostly stable in the dry state; thus, it makes salivary sample suitable for DNA testing. Identification of DNA in saliva by genetic profiling can be helpful in cases of sexual abuse and harassment. The foreign DNA usually remains in the victim's saliva for about 1 hour which could be of great piece of forensic evidence.[17]

1.6 Malignancy: Molecular markers for the diagnosis can be identified as changes in cellular DNA, altered mRNA transcripts and altered protein markers. That can be observed in salivary samples. [18,19]

1.7 Psychological condition: Saliva has been used to monitor therapeutic responses in the treatment of anxiety by measuring the salivary level of 3-methoxy-4-hydroxy phenyl glycol and also it has been used to measure post – traumatic stress disorder associated with wartime.[20]

1.8 **Renal disease:** Salivary phosphate has been widely used as a clinical biomarker for hyperphosphatemia. [21]

1.9 Viral infection: In a study performed by Oliveira et al., the measles virus-specific IgM was detected in the saliva. HIV diagnosis by detecting p24 antigens and antibodies against both HIV-1 and HIV-2. [22,23] Recent studies have shown that saliva-based testing for COVID-19 detection holds a promising alternative to the oropharyngeal and nasal swabs.[24]

1.10 Bacterial infections: Mycobacterium tuberculosis, can be detected in saliva by a polymerase chain reaction, H. pylori that binds to the salivary mucins (MUC-5B and MUC 7) can also be detected in saliva. Higher levels of these salivary mucins could be indicative of infection with H. pylori.[25]

Presently, many manufacturers produce saliva collections devices for obtaining stimulated and unstimulated saliva. [6] One of the newly emerging devices used along with the saliva collection device developed by the Oasis Diagnostics® known as Litebox Image Analysis Module (LIAM) which is a small hand-held saliva collection tool that can provide immediate quick results from its sample.

2. LITEBOX IMAGE ANALYSIS MODULE [LIAM]

LIAM is a compact portable device using VerOFy® technology. After the assessment of the saliva sample, the data can be forwarded to any Bluetooth device or a smartphone. This tool is light weight, easy to use, and is battery powered. Due to its unique features, it is easy to handle and can be used in remote locations [26] This tool mainly focuses on VerOFy® technology. It comprises of immune-chromatographic test strips that produces quick results of the collected saliva sample with effective delivery of results. The good thing about this tool is that it can be used even in far off inaccessible locations due to its easy to carry feature.

2.1 BENEFITS OF LIAM

LIAM is an extremely handy user-friendly device that could provide results within few minutes after the collection of the sample, non-invasive technique, Easily accessible even in inaccessible remote areas, assessment of various diseases from a single saliva sample, evaluation of various biomarkers present in the saliva, that could ease the health workers to diagnose the disease state at an early stage and access the disease status of the individual. This device is also capable of assessment of cortisol level of saliva, testosterone levels and biomarkers of various hormone.

2.2 EQUIPMENT OF LIAM MODULE

LIAM device is a fluorescent LFT strip reader that can analyse and transfer the information to another Bluetooth enabled device, VerOFy Cartridge that contains the lateral flow test strips, Super •SAL (Figure. 2) contains compression tube, absorbent pad that absorbs the saliva sample, Sample volume indicator that turns to red when adequate sample is collected, Sample collection tube (Eppendorf tube) where the saliva sample is stored and a Disposal pipette through which the sample is transferred to the test strip from the collection tube. (Figure. 1)

2.3 PROCEDURE FOR COLLECTION OF SALIVA SAMPLE

Patient is refrained from consumption of food, drinking, tobacco smoking or using mouth rinsing products for about 10 mins before the collection of the sample. The patient was asked to sit in an upright position and requested to pool the whole unstimulated saliva in the mouth, followed by placement of the Super•SAL collection device absorbent pad horizontally in the oral cavity (Figure 3a). After adequate saliva sample was absorbed the sample volume indicator turned red (Figure 3b), up to 2ml of saliva could be collected. Followed by the placement of absorbent pad into the compression tube in an upright position, the plunger is pushed slowly downward at a rate of not more than 2-3 drops/second (Figure 3c), which eliminates any additional unnecessary particulates that can compromise the Lateral Flow Test. The collected saliva sample is then transferred into the Eppendorf tube, the sample is inverted several times to ensure a homogenous mix, with the help of a disposable pipette. Filtered saliva is transferred from the Eppendorf tube to the right and left well of the cartridge about 7 drops and wait for about 20 minutes to let the sample flow in the LFT. [27]

The cartridge is ready to be analysed via LIAM. Results can also be forwarded to any Bluetooth capable smartphone or laptop.

2.4 PROCESSING OF THE SAMPLE

2.41 Lateral Flow Test (LFT) System

Saliva from the Super•SAL is examined with the LFT, the first section is a testing compartment that includes two LFT strip. The sample pad acts as a storage site that delivers saliva into the conjugate pad, that remains just below the housing sample well. The dried conjugate then uses saliva as a medium for its hydration. The free and conjugate bound analyte in the sample reacts with the europium fluorescent particle-based conjugates that are present in the VerOFy LFT. Later the reacted conjugate that is obtained from the conjugate pad are collected by the nitrocellulose membrane, then this membrane fixes the reacted product on the two capture zones that contain bands. The primary bands contain the restrained antibody for the analyte to be found in the given saliva sample. The second band contains a group of molecules that did not adhere to the primary bands. Due to the capillary action acting on the strip the fluid was continuously being pulled from the nitrocellulose membrane to the absorption pad. (Figure 4) [28,29]

2.42 LIAM

LIAM a fluorescent LFT strip reader that can analyse and transfer the information to another device with the help of Bluetooth connectivity. The Lateral flow test strip after exposure to the saliva sample, is then inserted to the module, that excites the nitrocellulose membrane to

ultra-violet light in a specific direction to ensure proper image analysis. The module then displayed the data in picogram per millilitre units and transferred the information to a Bluetooth enabled devices.

Shirtcliff et al.[28] conducted a study with the help of Lateral flow technology cortisol device that was able to measure the cortisol level in the saliva minutes after collection of the sample as compared to the traditional immunoassays that usually takes days to months for the cortisol results. In the study 29 adults were selected and their saliva sample were collected in the morning and afternoon by passive drool and Super•SAL™ Extra Collection Device and later the sample was assayed with the help of LFT and traditional enzyme-immunoassay. The study revealed a good correlation between the collection methods and the cortisol levels assayed with the help of LFT and enzyme-immunoassay.

Elizabeth et al. conducted a study to access the real time salivary cortisol using the LIAM device. Unstimulated whole saliva was collected from twenty-three participants and the results were evaluated using the device. The values were highly correlated with the salivary cortisol levels measured using a commercially available EIA kit. [30]

Hence this device is capable of providing real time results in very short time from the saliva sample using a small hand-held reading device on the field. However more research has to be done to better understand the feasibility and outcomes of this device in the field of salivary diagnosis.

3. FUTURE INVESTIGATIONS

Investigations that could be done with this device in the near future include Alzheimer's dementia, Idiopathic Parkinson's disease, Sleeping disorders, Mullerian-inhibiting hormone.[26]

4. Lateral flow immunoassay (LFIA) VS Enzyme-linked immunosorbent assay (ELISA)

ELISA has a higher turnaround time as compared to LFIA because of the lengthy procedure as the sample requires further preparation these significantly adds the turnaround time to days and weeks between the receipt of the sample and returning of the results. Whereas with LFIA it is much faster, the result is available within minutes on the spot.

There is a procedural difference between ELISA and LIAF, ELISA has a complicated procedure that requires specialized laboratory training and equipment to prevent error. It is also time-sensitive, technique sensitive. Whereas LFIAs are not complicated, requires a few steps, doesn't require any specialized training.

ELISA and LFIA also differ in their workflow. ELISA preparation steps include cycles of wash of the 96-well microplates to remove residues of the biofluids along with sample preparation steps that should be precisely done to avoid any error in the results. However, LFIAs do not require lengthy sensitive steps to purify the biofluid prior analysis, instead it uses simple, fast and short purification steps to ensure a clean specimen for analysis in LFIA test strip. For the test to be successful the biofluid must produce discreet bands on the strip at Test and Control region.

For ELISA large and specialized equipment is required for transportation, storage of sample and to obtain results. In contrast, LFIAs do not require high-tech equipment, can be stored in

room temperature. New generation LFIAs require 'readers' that has image analysis computer software and instrumentation that detects particles on the test strips. [29]

5. Recent advances in Lateral Flow Immunoassay

Lateral flow immunoassays are relatively new, but the field however continues to innovate and several advances have emerged within this novel field in recent years. There have been tremendous technological developments and improvements in the manufacturing of rapid, small-scale tests. Such as lab-on-a chip (LOC) systems have advantages like convenience, compact format and large-scale manufacturability, no requirement of high-tech labs. LOC systems are combined microfluidic devices, which look like a computer chip with microchannels, they work on the basis of microfluidics with a sensor for detecting the analyte it just requires a reader that could interpret the signal from the chip. These can be used for the detection of E. coli, H1N1, as well as DNA analysis.[31]

Another recent advance is the innovation in salivary LFIA application, Episcreen first clinically adopted saliva test for HIV following FDA clearance in 1997. Salivary LFIAs also have been used in the field of drug abuse testing and for various other salivary biomarkers it has potential in numerous applications in the future.[32]

A rapid multiple-biomarker panel can be adaptable enough to meet the specific needs of patients for clinical, research, and industrial contexts. In addition to providing the convenience and simplicity of a rapid salivary LFIA to the user, this technology can allow the users' personalized perception into their own health and body function.[29]

6. CONCLUSION

This paper provides a gist of salivary biomarker detection signifying the probable status of disease of an individual using a portable device LIAM. Therefore, LIAM is and will be an efficient portable saliva detection substitute for use in cities as well as specially in remote far off areas where latest technologies like advanced equipment as well as infrastructures like highly equipped laboratories are not easily accessible. Not many research papers are published yet regarding this device emerging a need for more research for near future.

REFFERENCE

1. Malathi N, Mythili S, Vasanthi HR. Salivary Diagnostics: A Brief Review. ISRN Dent. 2014;1(1):1-8.
2. Madalli VB, Basavaraddi SM, Burde K, Horatti P. Saliva-A diagnostic tool. IOSR J Dent Med Sci. 2013;11(6):96-99.
3. Costa PT, Chauncey HH, Rose CL, Kapur KK. Relationship of parotid saliva flow rate and composition with personality traits in healthy men. Oral Surg, Oral Med, Oral Pathol. 1980;50(5):416-422
4. Roi A, Rusu L, Roi C, Luca R, Boia S and Munteanu R. A New Approach for the Diagnosis of Systemic and Oral Diseases Based on Salivary Biomolecules. Dis Markers.2019;3(4)1-11.
5. Shah S. Salivaomics: The current scenario. J Oral Maxillofac Pathol 2018;22(3):375-381

6. Khurshid Z, Zafar MS, Najeeb S, Zohaib S. Human Saliva: A Future Diagnostic Tool. EC Dent Sci.2016; 3(6):635-636.
7. Saikia J, Pachipulusu B, Govindaraju P, Das D. Assessment of superoxide dismutase levels in saliva among tobacco and non-tobacco users - A cross sectional study. J Adv Med Dent Scie Res.2019;7(12): 66-72.
8. Malhotra T, Sachdeva A, Bhateja S, Arora G. Salivary biomarkers as a diagnostic tool. J Surg Allied Sci 2019;1(1):1-4.
9. Martina E, Campanati A, Diotallevi F, Offidani A. Saliva and oral diseases. J. Clin. Med. 2020;9(2):466.
10. Malamud D. Saliva as a diagnostic fluid. Dent Clin North Am. 2011;55(1):159-178.
11. F. Ahmadi Motamayel, P. Davoodi, M. Dalband, and S. S. Hendi. Saliva as a mirror of the body health. DJH Journal 2010; 1(2):1–15.
12. M. Greabu, M. Battino, M. Mohora et al., Saliva—a diagnostic window to the body, both in health and in disease. J Med Life. 2009;2(2)124–132.
13. S. Mittal, V. Bansal, S. Garg, G. Atreja, and S. Bansal. The diagnostic role of Saliva—a review. J Clin Exp Dent. 2011;3(4): e314–e320.
14. H. H. Chan, Z. H. A. Rahim, K. Jessie, O. H. Hashim, and T. B. Taiyeb-Ali, Salivary proteins associated with periodontitis in patients with type 2 diabetes mellitus. Int. J. Mol. Sci. 2012;13(4):4652–4654.
15. Adam DJ, Milne AA, Evans SM, Roulston JE, Lee AJ, Ruckley CV, Bradbury AW. Serum amylase isoenzymes in patients undergoing operation for ruptured and non-ruptured abdominal aortic aneurysm. Journal of vascular surgery. 1999;30(2):229-35.
16. Mittal S, Bansal V, Garg S, Atreja G, Bansal S. The diagnostic role of Saliva — A Review. J Clin Exp Dent. 2011;3(4):e314-20
17. N. Kamodyova, J. Durdiakova, P. Celec et al. Prevalence and persistence of male DNA identified in mixed saliva samples after intense kissing. Forensic Sci. Int. 2013;7(1)124–128.
18. H. Tang, Z. Wu, J. Zhang, and B. Su Salivary, lncRNA as a potential marker for oral squamous cell carcinoma diagnosis. Mol. Med. Rep. 2013;7(3)761–766.
19. J. M. Yoshizawa and D. T. W. Wong, Salivary microRNAs and oral cancer detection. Methods Mol. Biol. 2013;9(36):313–324.
20. Yamada S, Yamauchi K, Yajima J, Hisadomi S, Maeda H, Toyomasu K, Tanaka M. Saliva level of free 3-methoxy-4-hydroxyphenylglycol (MHPG) as a biological index of anxiety disorders. Psychiatry Res. 2000;93(3):217-23.
21. V. Savica, L. Calò, D. Santoro, P. Monardo, A. Granata, and G. Bellinghieri,. Salivary phosphate secretion in chronic kidney disease. J. Ren. Nutr. 2008;18(1): 87–90.
22. S. A. Oliveira, M. M. Siqueira, D. W. G. Brown, L. A. B. Camacho, T. Faillace, and B. J. Cohen, Salivary diagnosis of measles for surveillance: A clinic-based study in Niteroi, state of Rio de Janeiro, Brazil. Trans. R. Soc. Trop.1998;92(6):636–638.
23. K. P. Delaney, B. M. Branson, A. Uniyal et al. Evaluation of the performance characteristics of 6 rapid HIV antibody tests. Clin. Infect. Dis. 2011;52(2):257–263
24. H Altawalah, F AlHuraish, WA Alkandari, S Ezzikouri. Saliva specimens for detection of severe acute respiratory syndrome coronavirus 2 in Kuwait: A cross-sectional study. J. Clin. Virol. 2020; 132:104652.
25. D. G. Silva, R. H. Stevens, J. M. B. Macedo et al. Higher levels of salivary MUC5B and MUC7 in individuals with gastric diseases who harbor Helicobacter pylori. *Arch. Oral Biol. 2009;54(1):*86–90.

26. Oasis Diagnostics®. 2020. *Verofy® & LIAM™ - Oasis Diagnostics®*. [online] Available at: <https://4saliva.com/products/verofy/> [Accessed 26 April 2020]
27. Filgen.jp.2020.[online]Availableat:<https://filgen.jp/Product/Bioscience4/Oasis/SSAL-601.pdf> [Accessed 26 April 2020].
28. Shirtcliff EA, Buck RL, Laughling MJ, Hart T, Cole CR, Slowey PD. Salivary cortisol results obtainable within minutes of sample collection correspond with traditional immunoassays. Clin Ther. 2015;37(3):505-514.
29. Miocevic O, Cole CR, Laughlin MJ, Buck RL, Slowey PD and Shirtcliff EA. Quantitative lateral flow assays for salivary biomarker assessment: A review. Front. public health.2017;5(1):133.
30. Shirtcliff, Elizabeth & Slowey, Paul & Hart, Tom & Buck, Robert. (2013). Real-time salivary cortisol enhances the match of an individual's stressor with their stress reactivity.
31. Erickson D, O'Dell D, Jiang L, Oncescu V, Gumus A, Lee S, et al. Smartphone technology can be transformative to the deployment of lab-on-chip diagnostics. Lab Chip (2014) 14(17):3159–64
32. Chong H, Koo Y, Collins B, Gomez FA, Yun Y, Sankar J. Paper-based microfluidic point-of-care. diagnostic devices for monitoring drug metabolism. J Nanomedine Biotherapeutic Discov (2013) 3(1): e122.

FIGURE LEGEND:

Figure 1: Elements of LIAM device. [11]

Figure 2: Components of Super•SAL device. [11]

Figure 3a: Super•SAL collection device, absorbent pad placed horizontally. [12]

Figure 3b: Sample volume indicator turns red. [12]

Figure 3c: Place the absorbent pad into the compression tube, slowly push the plunger downward at a rate of not more than 2-3 drops/second. [12]

Figure 4: Lateral Flow Strip. [13]

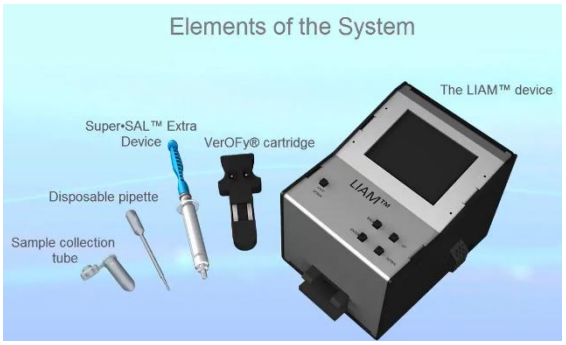

FIGURE: 1

FIGURE: 2

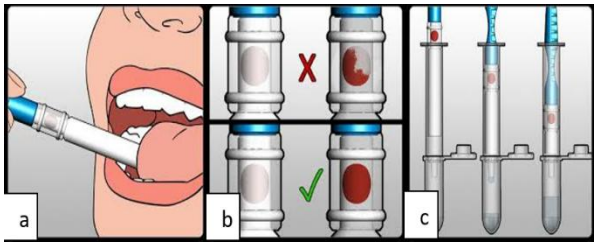

FIGURE: 3 (a,b,c)

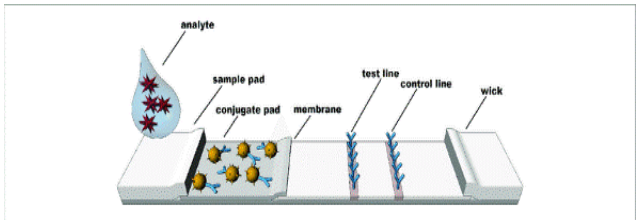

FIGURE: 4