

Role of Saliva in Point of Care Technology

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ABSTRACT

Introduction: Saliva has been used as sample for point of care monitoring. In addition to its feasibility of use, the advantages of saliva when compared to other body fluids, the new technically advanced methods to detect the biomarkers of interest in saliva rendered the point of care testing (POCT) for wider applications.

Significance of POCT: An accurate, quick and portable POCT is ideally required for hospital, laboratory and personal applications. Together with point of care diagnostics, the scientifically validated numerous upcoming biomarkers will extend their applicability for real time health tracking and personalized precision medicine in different areas of health care systems. The utilization of salivary POC analytical procedures is perhaps in its emerging stage.

Conclusion: This paper summarizes the overall perception regarding the salivary point of care testing and its applications.

Key words: saliva, point of care, dentistry

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INTRODUCTION

Early detection of disease can enhance patient's quality of life by avoiding its progression and further complications. Saliva has been used as a biomarker diagnostic tool for variety of clinical purposes. The word salivaomics depicts the collective learning of saliva and its components, tasks, and associated methods. A framework for the use of oral fluid as a diagnostic medium has been developed by the National Institute of Dental & Craniofacial Research (NIDCR). Further invention of different identification. Newer developments in the methodology tools and techniques and the rendered saliva as a possible sample for biomarker. Validated biomarkers can be used to build POC devices that make the application faster and easier. With different collecting devices, the saliva can be collected either in a stimulated or unstimulated manner and can be used either directly or after preprocessing according to the manufacturers direction. The trained staff were also required at times to operate POC devices.^{1,2} While traditional approaches have detected broad range of salivary biomarkers, very few have been used in point of care applications.

Salivary biomarkers and liquid biopsy

The salivary molecular profile studies would include salivary proteomics, salivary transcriptomics, salivary genomics, salivary metabolomics; salivary microbiomics and other miscellaneous marker studies.² The discovery of these biomarkers have been utilized in variety of clinical applications and further helps in the development of salivary POCT techniques.

In order to develop POCT using saliva, it is essential to recognize a feasible, accurate, and validated salivary bio-

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markers with scientific credential. Various studies reported the significant role of salivary biomarkers in screening and monitoring of systemic diseases, oral diseases and pharmacotherapeutic purposes.

Another important area of interest for POC development is liquid biopsy. The two important components in the liquid biopsy aiming in the biofluid that have been related to cancer is circulating tumour cells (CTC) which is introduced into the bloodstream by the primary tumour and the circulating tumor DNA (ctDNA) that could be processed and identified from the body fluid. The low concentrations of CTCs among blood cells need a huge volume of sample size or highly sensitive detective methods to detect the CTC. In contrast, ctDNA targets can simply be separated through conventional DNA

extraction techniques. The membrane-bound structures called extracellular vesicles (EVs) which encapsulate ctDNA, it is assumed that ctDNA are constantly witnessed in biofluid and are discharged by cells into the circulation.³ The salivaomics and liquid biopsy are indispensable research fields for the emerging salivary point of care.

Salivary point of care

Point of care testing facilitates the rapid screening or monitoring of chronic conditions which assists in quick and easy personal monitoring and for clinical decision making. The point of care testing could be either minimally invasive or non-invasive. The well known point of care testing using blood is the rapid glucose monitoring system and by urine is the rapid pregnancy test kit. In addition, POCT is widely used to detect drug abusers using common body fluids or breathing.⁴ In dentistry, the POC varies from light based devices for diagnosing abnormal oral mucosa, nicotine breathe analyzer, laser units, endo motor and computer aided devices for impression making and prosthesis fabrication etc. The ultimate aim of point of care testing is the shifting of the test from the conventional laboratory to clinic or home without compromising the accuracy and standard and it also saves the processing time. Utilizing saliva as point of care testing has many applications in diagnosing and monitoring the conditions like infectious diseases, cancers and genetic based illness etc.

WHO guidelines of POCT

The guidelines include affordability, sensitivity, specificity, user-friendly, robust, rapid, equipment free and deliverable for the needy persons with the abbreviation spells "ASSURED". Thus it allows the test results could be obtained within 30 minutes of times in resource poor settings.⁵

1. Advantage of saliva in POCT

Saliva and its other associated fluids like oral rinse, GCF, PISF (peri-implantitis sulcular fluid) has many advantages of easy obtainability, self collection, less contamination and non-invasive sample collection. Its feasibility makes repeated collection of sample without much discomfort. The diverse salivary biomarkers like enzymes, proteins, metabolites, genetic and epigenetic markers, micro organisms, drugs, chemicals/elements and other miscellaneous markers are now widely applicable in diagnosing oral and systemic diseases, disorders

and tumors.⁶ Salivary POC testing devices are obtainable for a variety of components; its efficacy ranges from hospitals, outpatient clinics to personal care.

2. Limitations of salivary POCT

The presence of various biomarkers depends upon its intracellular location, molecular size, salivary flow characteristics and other factors and the POCT depends upon the collection of saliva, storage and other pre-processing methods. The particular biomarker /POCT should be reliable, sensitive and specific for valid clinical application.⁵

3. Various methods in POCT

In addition to biomarkers, systems such as biochip and biosensor also believed discrete diagnostic methods. The other reported systems are Biological Micro-Electro-Mechanical Systems (BioMEMS)⁷ and Nano electromechanical systems (NEMS).⁷

Lab-on-chip (LoC) system facilitates superior biological sample processing, manipulation and analysis in microscale fluidic devices. LOC-lab on chip-Microfluidic technology can be connected with handy units example; pumps, reactors and valves. The designing methods and selection of materials of microfluidic devices depends upon their final applications.

Recent available POCs can be distributed in the form of small, mobile and wearable POCT. The sample handling platforms would be either lateral flow assay/or microfluidic based. The signal transduction is via optical or electrochemical methods.

The smart phone based POCT are listed in Table 1.¹⁸ Paper based POC immunoassays comprised of Lateral Flow ImmunoAssay; Microfluidic paper based analytical devices (μ PADS) and nanoparticles.⁹

EFIRM-Electric field induced release and measurement

The salivary exosomes are extracellular vesicles and this nanostructure information could be utilized for the diagnosis of diseases in this method. This approach can be incorporated with magnetic electrochemical methods.¹⁰

4. Applications of salivary POCT

The applications and development of POCT depends upon the validation of the particular POCT in comparison to other biofluid/tissues and other conventional lab methods of identification and quantification in different cohorts. Recent POCT

Table 1:³ The smart phone based PCOT

Calorimetric
Fluorescence
QUASR : Quenching of unincorporated amplification signal reporters
BART-LAMP : Bioluminescent assay in real-time and loop-mediated isothermal amplification
RT-LAMP : Reverse-transcription loop-mediated isothermal amplification
Electrochemical
Screen -printed electrode

Table 2:²⁵ Applications in Dentistry

Conditions	Biomarkers
Oral cancer	IL-8, IL-8mRNA
Periodontal diseases	Proteins (Dipeptidyl peptidase etc.), metabolites, DNA
Sjogren's syndrome	Salivary anti-Ro60 and anti-Ro52 Antibody Profiles
Smoking/tobacco use	Salivary nicotine metabolites



have come up with the combination of biomarker identification which has greatly improved the time and diagnostic accuracy. The integration of scientifically validated biomarkers and new technical advancements certainly renders the salivary POCT possible as an economical device for different clinical/personal monitoring purposes.

Presently, two categories of salivary POC are reported that comes under single biomarker detection-based and multiplexing detection-based devices.^{5, 6, 11, 12} Table 2 summarizes the oral condition and the respective biomarkers. These biomarkers are utilized for POCT for diagnosing the same.^{11, 12}

The current areas explored using saliva for POC includes the screening of infectious diseases by Dentognostics (Germany) lab-on-a-chip technology and OraSure Technologies using lateral force-based chromatography test strips and for detecting multiple biomarkers for cancer screening and diagnosis within a minimal timeframe by UCLA SDxPOC. The sensor and cartridge has to be placed in by the user and by pushing the menu button, the readable results are displayed once the testing is complete.

Other POC devices like Oral Fluid Nano Sensor Test (OF-NASET) for the detection of oral cancer, OraRisk for HPV test are few examples of POCT using saliva. In addition, lab-on-a-chip (LOC) and Integrated Microfluidic Platform for Oral Diagnostics (IMPOD) is used to discover periodontitis, and for detecting sjogren's syndrome Luciferase Immunoprecipitation Systems (LIPS) has been used.^{11, 12}

Table 3 summarizes the systemic condition and their respective salivary biomarkers.¹¹⁻¹⁶ These are used in POCT for

the diagnosis of systemic conditions. Salivary α -Amylase (sAA) biosensor system and Label-free chemiresistor immuno-sensor is used for detecting stress associated conditions. Oraquick devices have been employed for detecting HIV and Hep C. The colorimetric and fluorescent assays that permits DNA and RNA detection in case of HPV associated cancers and diseases. For myocardial infarction Luminex, lab-on-a-chip methods are used. Fiber-optic microsphere-based antibody array is employed for detecting asthma and COPD and Optical fiber microarrays for end stage renal disease. Sexually transmitted infections (STIs) would be diagnosed with Oral STI point-of-care (POC) system. Programmable bio-nanochip (P-BNC) system is used for cardio vascular disease diagnosis. For detecting cancer Programmable bio-nanochip (P-BNC) system, 2D nanomaterials are used.^{11, 12}

EFIRM technology has been used to detect EGFR mutations in non-small cell lung cancer. Recently, a lung cancer diagnostic kit is developed for the detection of exosomal miRNA using lateral transfer flow test strips.^{12, 13} The immunochromatographic strip can be utilized as POCT for diagnosing pepsin in laryngopharyngeal reflex.¹⁴ Acoustofluidic micromixing enabled hybrid integrated colorimetric sensor has been described for salivary potassium identification.¹⁵ In addition, several studies suggested salivary microfluidic paper-based analytical devices (μ PAD) for identifying salivary glucose, α -amylase, lactate, nitrite and phosphate for different clinical applications. It has been suggested that SARS-CoV-2 RNA, viral load is abundant in saliva and salivary glands. Therefore, the development of salivary POCT for rapid mass/or repeated screening will benefit from the current pandemic situation especially in low resource settings.^{12, 16} However, the WHO is not yet recommending salivary POC as diagnostic procedure for the Covid-19 disease.

Currently, a number of salivary biomarkers have been documented in relation to dentistry to diagnose chronic myofascial pain, TMJ disorders and burning mouth syndrome, etc. A recent systematic review documented the association of salivary markers with different systemic malignant tumors, neurologic diseases and inflammatory/metabolic/cardiovascular diseases. The use of salivary markers in the identification of different neurodegenerative diseases and certain viral infections and further confirmation in the identification of these salivary biomarkers contributes to a high-throughput salivary POC systems and to the concepts of economic and simple utility.^{12, 16, 17, 18}

CONCLUSION

The collaboration of interdisciplinary research approach brings outmost advancement in development of POC technology which results in easy, quick and feasible products for the well being of the human health. The salivary POCT test will also give a gateway for the future advances in health science and function as an alternate choice to other body fluid diagnostics. The knowledge sharing between translational researches with clinical research will also result in early gain in patient care. The clinical effectiveness of salivary POC as a user friendly, effective and widely available technology will certainly be enhanced by further exploration of simpler devices and methods using saliva in POC. Standardized collection of saliva, pre processing procedure and storage condition protocols are essential factors for precise performance. Salivary point of care

Table 3:^{2,5-9} Applications in systemic states

Systemic conditions	Biomarkers
Stress associated conditions	A amylase, cortisol
Hepatitis	Hep c
AIDS	HIV
HPV	HPV
Acute MI and cardiovascular disease	Myoglobin, C-reactive protein, and myeloperoxidase
Diabetes	Glucose
Chronic Obstructive Pulmonary Diseases	Cytokines
End stage renal failure	pulmonary inflammation biomarkers, NO ₂ and uric acid
Sexually transmitted infections	Chlamydia and Gonorrhoea
Systemic cancer	ca125, cea, Psa, Her2-neu
Lung cancer, non-small cell lung cancer	Exosomal RNA, epidermal growth factor receptor (EGFR) mutation
Laryngopharyngeal reflux	Pepsin
Potassium level	Potassium



will play a major role in the future and serve as a link between the field of medicine and dentistry in relation to patient care.

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