International Journal of Biomedical Research
ISSN: 0976-9633 (Online); 2455-0566 (Print)
Journal DOI: https://doi.org/10.7439/ijbr
CODEN: IJBRFA

Original Research Article

Saliva: A systematic review on its diagnostic utility

Neeharika Mortha[*1], Divya Uppala[1], Nandita Rani Kothia[2], Sumit Majumdar[1] and Sravya K[1]

[1]*Department of Oral & MaxilloFacial Pathology, GITAM Dental College & Hospital, Visakhapatnam, 530045, India*
[2]*Department of Public Health Dentistry, SIBAR Institute of Dental Sciences, Takkelapadu, Guntur-522509, India*

QR Code

***Correspondence Info:**
Dr. Neeharika Mortha, (MDS)
Post Graduate student,
Department of Oral & MaxilloFacial Pathology,
GITAM Dental College & Hospital, Visakhapatnam, 530045, India

***Article History:**
Received: 12/06/2017
Revised: 29/06/2018
Accepted: 08/01/2018
DOI: https://doi.org/10.7439/ijbr.v9i1.4233

Abstract

Introduction: A paradigm shift in recent years has led to the consideration of the oral cavity (and, thus, oral disease) not in isolation but as a component integrated with systemic physiology, important in maintaining systemic health and reflective of systemic disease. Saliva has been studied extensively as a potential diagnostic tool over the last decade due to its ease and non-invasive accessibility along with its abundance of biomarkers.
Aim & Objective: This is a systematic review of the studies published in past 15 years in electronic databases regarding the utility of saliva as a diagnostic tool.
Materials & Methods: Data collection from electronic databases, followed by data extraction and management.
Results & Conclusion: Of 85 studies considered among 510, represent the evolution of saliva in screening neoplasm, with 34 different analytes detected in this population followed by 22 in metablic disorders and the least being in systemic disorders. Most abundantly applied technique being ELISA, common analyte being Immunoglobulins. The application of high throughput proteomic techniques in various studies in the past decade has led to the discovery of new biomarkers.
Keywords: Oral, Analytes, Biomarkers, Database.

1. Introduction

In day- to- day life, the service delivered by saliva is unrecognized. Saliva protects the teeth and the oro-oesophageal mucosa through a number of mechanisms. Besides maintenance of the integrity of these tissues, saliva also has multiple functions in relation to digestion in the upper gastrointestinal tract. Lubrication of oral surfaces, tooth mineralisation, buffering and antimicrobial activity are some more benefits of saliva [1]. This Saliva, known as oral fluid is derived predominantly from salivary glands primarily parotid, submandibular and sublingual glands apart from the numerous minor salivary glands.

It is believed that changes in saliva are indicative of wellness of our health [5]. Saliva was considered over other possible diagnostic biofluids, not only for the presence of targeted specific molecular constituents, also due to its ease of use as a practical method of collection. This makes saliva a good alternative over blood for diagnosis, interpretation and evaluation of various analytes, also for numerous other health surveillance programmes. The areas where salivary diagnostics can be applied include the fields of medicine, dentistry, pharmacotherapy, epidemiology & bioterrorism.

In the present scenario worldwide, the identification of susceptible individuals at risk from diseases like oral cancers, precancerous lesions and conditions, other oral diseases and certain systemic illness, represent a challenge to both clinicians and researchers [4]. Several studies have contributed vast information on biomarkers that can be used to detect the early stages of disease development from saliva which paved way to the development of newer methodologies, resulting in the emergence of salivaomics to its current state. The term saliva omics was coined in 2008 to reflect the rapid development of knowledge about various "omics" constituents of saliva [2,3]. This systematic review aims to collect published data on studies using Saliva as a diagnostic tool, in past 15 years and determine the significant techniques involved in evaluating the prominent, among the number of analytes detected in saliva with graphical representation.

2. Materials and Methods

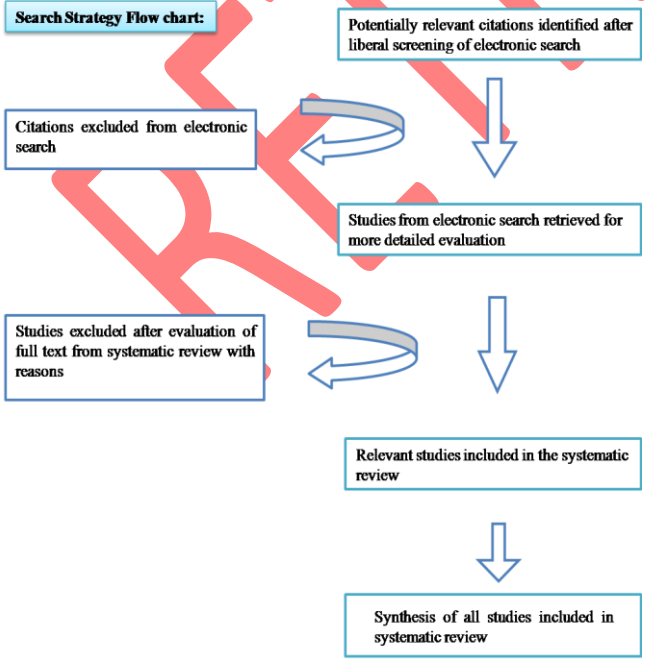
2.1 Data collection

- Study identification included initially electronic databases: GOOGLE SCHOLAR & MEDLINE with key word – "saliva as a diagnostic tool"
- The electronic databases PubMed & Science Direct were then searched for articles published in English since 2002 until 2017 inclusive.
- Two independent reviewers extracted information regarding study design, study population, interventions, outcome measures, results and conclusions for each article. The relevant studies were identified following a comprehensive literature search.
- Assessment of the quality of the included studies was done to minimize the risk of selection bias.
- The mesh terms were "saliva" and "diagnostic tool", "Saliva", "diagnostic".

2.2 Data extraction and management

- Data was extracted from all the included studies. In a Microsoft Word document, the extracted data was entered electronically to facilitate the summary and analysis. This included (1) study details (study year, study design, study aim);(2) study and population characteristics(3) intervention (4) outcomes (5) results
- Data extraction was done by two reviewers independently, to increase the accuracy, and any disagreements were resolved by consensus or by involving a third reviewer.
- Collected information was entered in a spread sheet to facilitate graphical representation.

Figure 1: Search strategy flow chart



2.3 Inclusion criteria

- The review includes studies published between 2002 to present date on electronic databases "Pubmed", "Medline", "Science Direct" & "Biomed Central".
- Search from all previous studies based on database entry date plus publication date (to ensure continuity) till date was included.
- Only articles published in English were included.

2.4 Exclusion criteria

- Studies that do not attempt to measure health impacts on humans.
- General discussion papers not presenting data.
- Any article that was obviously unsuitable was excluded in the early stages or the search (for example, on the basis of abstracts and titles presented in electronic catalogues).
- We excluded review articles, editorials, consensus statements and opinions.

3. Results

Considering the inclusion and exclusion criteria for 510 studies, 85 studies were taken for this systemic review. The studies with detailed data on sample size, methodologies, and results, values of significance, sensitivity and specificities were considered. Among 85 studies majority were cross sectional studies, with cohort studies and non-randomized trials among the least. On evaluating the collected data, it was interpreted as:

- Majority of saliva based studies are conducted in Neoplasm followed by infections, metabolic disorders, periodontal and systemic diseases. [Figure 2]
- Highest numbers of studies were conducted in the year **2015**. [Figure 3]
- The most commonly applied method was **(ELISA)** Enzyme Linked Immunosorbent Assay followed by Real time (PCR) Polymerase Chain Reaction, Quantitative PCR, Glucose Oxidase Peroxidase methods.[Figure 4]
- The studies compare and quantify the values of analytes in saliva to **serum** commonly, followed by plasma majorly.
- Abundantly studied analyte were **Immunoglobulins** in various infections
- Among the 85 studies, nearly **34 different analytes** have been detected in saliva of patients with neoplasm, 22 in metablic disorders and the least being in systemic disorders.[Figure 5]
- **80%** of the studies emphasize the utility of **whole unstimlated saliva.**
- Preferred method of collecting the saliva among various studies remained the **Passive drool method.**
- Among the studies saliva was Compared and Correlated with **10** different mediums of interest. [Figure 6]

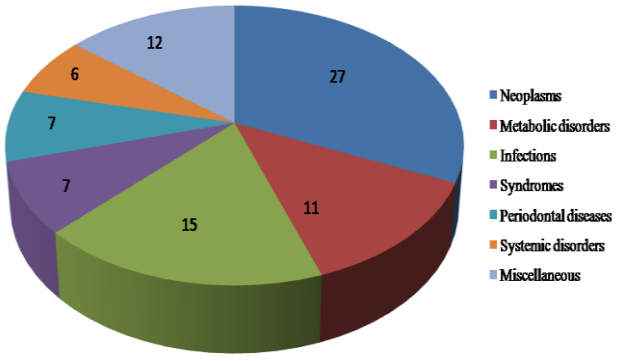


Figure 2: Number of studies in diagnostic area

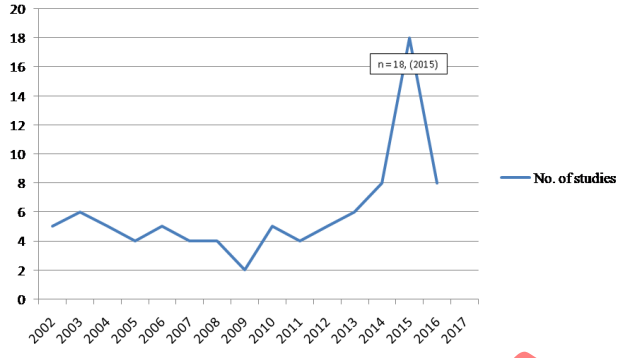


Figure 3: Number of studies

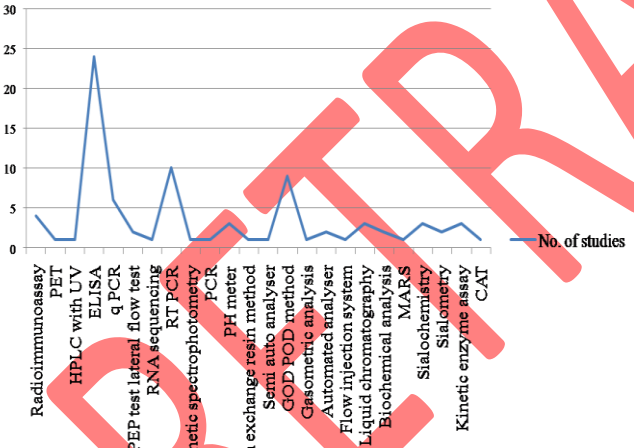


Figure 4: Applied Techniques

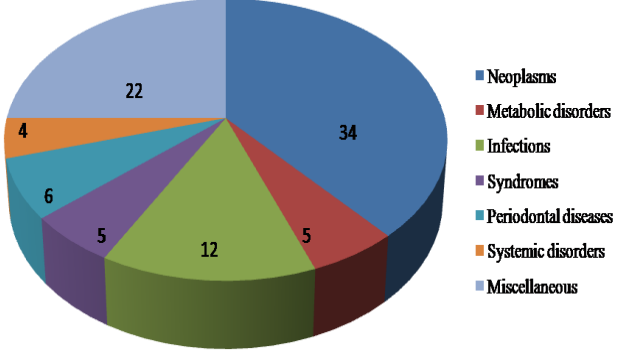


Figure 5: Number of analytes detected

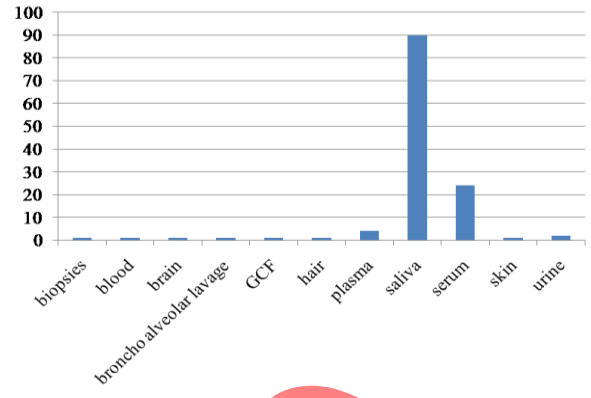


Figure 6: Comparison and Correlations

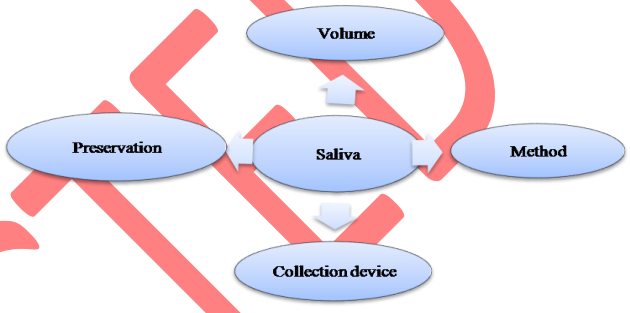


Figure 7: Considerations while using saliva as a diagnostic tool

4. Discussion

Saliva an oral fluid is derived from 3pairs of major salivary glands (accounting for about 90% total fluid secretion) and from the minor salivary glands (accounting 10% of total volume). Under resting state, 2/3rds of volume of whole saliva is produced by submandibular glands, upon stimulation parotids account almost half of the whole saliva volume in the mouth and a small percentage of unstimulated or stimulated whole saliva is contributed by sublingual glands with minor salivary glands making only a small contribution to total saliva volume. Salivary fluid is 99% water and <1% dry matter (proteins and salts) [1,5]. Daily salivary production ranges between 0.5-1.5 litres and is hypotonic relative to plasma. Blood supply to major salivary glands is quintessential for salivary production since the fluid in saliva originates from capillaries and the interstitial fluid. Many biochemical signaling processes are involved in the secretion of electrolytes, water and exocytotic release of proteins from acini[1].

4.1 Advantages

The saliva in being useful for diagnostic purposes and its obvious benefits over serum/plasma is dedicated to its practicality that includes ease, being non-invasive and it has a non-stressful manner of collection. For some illegal drugs when it is only detection rather than quantification, saliva provides a simple screening assay for illegal drugs like cocaine and marijuana [1,9]. In another arena, saliva is used to trace the pathogens scores, various elements, evaluation of analytes of interest and drug monitoring.

Thus, exploring various facets of saliva by many researchers, it can interfere beyond reasonable doubt that saliva is valuable for qualitative and quantitative assessment [5,7,8].

4.2 Application

With an expanding range of advanced technology and computational tools, today a large number of studies have been performed using saliva at a scale unrealized previously. This has served for the quantification, detection and analysis of a number of analytes and parameters, advancing towards the discovery and validation of salivary biomarkers [Table 1].

Table 1: Applications

S. No.	Diagnosis areas	Most common analytes detected
1	Neoplasm	CA19-9, sol, CD44, LDH Isoenzymes, MMP-9, (IL) Interleukins 6, IL 8 IL 1 alpha, HPV16, Cu
2	Metabolic Disorders	Glucose, Total Protein
3	Infections	HIV-Ab, Hepatitis B Surface Antigen, IgG, IgA, IgM
4	Syndromes	Cortisol, Flow Rate, Na, Cl
5	Periodontal diseases	Nitric Oxide
6	Systemic disorders	Corticol, Glucose, Alpha Amylase, mi RNA
7	Miscellaneous	Cortisol, Urea

To avail the benefits of saliva, protocols for optimal collection, processing, and storage procedures are to be followed (Figure 7). Saliva collection varies for individual participant groups in terms of volume and method of obtaining. Among the methods used (Resting /Passive drool, swab systems, spitting) the gold standard for many researchers has been, the drooling method, because it provides the purest sample possible and allows researchers to "biobank" samples for future testing[6]. Depending upon the type of saliva necessary for particular analyte detection, the saliva types include:

- Whole unstimulated (mixed saliva).
- Specific glandular saliva (parotid / submandibular / sublingual saliva alone)
- Stimulated saliva (by chewing paraffin block/ citric acid stimulation etc).

4.3 Interpretation

There are several systematic reviews presenting the diagnostic utility of saliva, with descriptions on the techniques applied for salivary analyte detection in health and disease, their application in salivary research and discussion on various "omics" (proteomics, genomics, metabolomics) of saliva. In fact, more than 50% of the available literature (2002-2017) is related to "salivary biomarkers" and "salivary diagnostics" in PubMed. This is a systematic review on the areas of diagnostic interest using saliva as diagnostic medium, the most common analytes evaluated, the most commonly applied techniques for detection of analytes in saliva and the most chosen methods of processing and storing the saliva by various researchers.

Among the 85 studies from 510 on the basis of inclusion and exclusion criteria, a maximum number of studies on utilizing saliva as a tool were undertaken in the year 2015. A time line of 15 years depicts a steady rise in the number of studies published after 2010 to 2015 till date, with least number of publications between 2009 and 2010. The studies attempted between 2002 and 2015 concentrate on detection of analytes marking the early diagnosis of neoplasm chiefly of head and neck [14-16], mainly oral squamous cell carcinoma. Among the 24 studies in subjects with neoplasms majority were conducted in oral squamous cell carcinoma followed by tongue cancer and parotid tumours the least. Of the 24 selected, 19 studies compared the levels of desirable analyte in comparison to premalignant lesions comprising oral leukoplakia commonly, followed by oral lichen planus and oral submucous fibrosis.[14,16,25] A significant correlation was established with serum levels and also, their correlation with histopathological grades of disease manifestation has been presented in maximum number of studies. It was conclusive from that group of studies that saliva can be used as a tool not just for diagnosis, but to monitor prognosis. In some of the studies there was not much significant difference in the estimation in saliva and serum. It can be inferred that saliva can be used as an adjunct tool. A small number of studies were conducted in subjects with syndromes associated with oral lesions. The studies published in 2015 dealt with the diagnostic potential of saliva in monitoring diabetes. Among 14 studies attempted on diabetic subjects comprising both type 1 and 2, 95% studies were conducted using the (GOD POD) Glucose Oxidase and Peroxidase Method with significant results [26]. From 2015 onwards there was a breakthrough in the application of saliva as diagnostic tool in subjects with systemic illness (like levels of cortisol in depression), in studying post treatment changes in transplant patients (like renal transplant), implant placement (in periodontal diseases) and therapeutic drug monitoring [20,21].

In the first 8 years analysis of protein expression was done at a larger pace that differentially expressed in disease and health [19,22]. Using specific protein biomarkers with techniques like enzyme linked ELISA, radioimmunoassay, PCR dominated this part of time zone [22]. A maximum number of studies published concentrated on proteogenomics that included chiefly detection of m RNA, IL-1α, IL-6, IL-8, (VEGF) Vascular Endothelial Growth Factor-a, (TNF) Tumor Necrosis Factor-α, immunoglobulins and enzymes like C-reactive proteins in saliva and established significant results [14-16,22]. Proteomics has steadily gathered momentum over the past decade with the emergence of high-throughput proteomic

technologies like Mass spectrometry based methods and microarrays. In the later years, metabolomics gained attention from many areas of research that includes evaluation of metabolites and end products in saliva. Though earlier studies were attempted to detect trace and major elements [18], their significant role in a particular diagnosis of interest was not appreciated in comparison to blood. In studies conducted in other body fluids taken as standard like levels of Urea, Creatinine in urine vs. saliva, it was inferred that saliva can be used as an adjunct diagnostic tool. Moreover in recent past, studies were published on detection of various metabolic end products like lactic acid, pyruvic acid, sialic acid in saliva of subjects with neoplasms including both malignant and pre malignant lesions and conditions, cardiovascular diseases [23,24] etc. Significant results were established in this field with respect to the gold standard considered. Although extensive research is required to consider the most effective means of detection in saliva among all the existing methods like gel electrophoresis, liquid chromatography, kinetic assays and spectrophotometry. To draw further conclusions as to which analyte or biomarker is of significance in early detections a vast number of studies are to done with respect to each analyte followed by meta analysis.

Although limitations in processing and storing of saliva exist, interest in saliva as a screening medium has grown in the last 10 years. Since the time of saliva collection to storage, the major hindrance being contamination with food debris, microorganisms and blood remains constant. With some researchers highlighting the contamination of saliva with gingival crevicular fluid, poses major disadvantage in conducting studies with both samples. Also whole unstimulated saliva is preferred over stimulated saliva to avoid interference with stimulants used. Depending on the type of analyte the choice of collection differs. With citric acid as stimulant or polyester rolls has shown to affect testosterone and cortisol concentrations in immunoassays. The commercial saliva collection kits used in studies also pose limitations. Saliva samples should be refrigerated at 4°C for processing within 3 to 6 hours after collection. (SAA) Serum Amyloid A Protein, (UA) Urine Protein, total protein and low molecular weight antioxidants remain stable up to two weeks at -20°C while Cortisol concentrations were not affected when stored at -80°C for one year or at 5°C up to 3 months. Otherwise, concentrations of cortisol were decreased approximately 10% in samples stored for 30 days at room temperature [11-13]. Addition of sodium azide, a preservative to saliva may cause interference in immunoassays. To avoid this pre-analytical treatment as used for blood or urine samples must also be applied for saliva [11,12].

5. Conclusion

The emergence of saliva as a diagnostic medium evoked many research questions from its practility to application. The published studies have inferred the role of saliva in screening various diseases with the presence of specific analytes. The application of techniques like ELISA, PCR, Microarrays etc. in saliva when compared to serum faces challenges in processing and storing. Also, a good number of studies are to be done using newer methodologies with any of the chosen particular analyte, in the field of diagnostic interest to achieve standardisation, and in terms of evaluation the specificities and sensitivities of the techniques and methods used are accurate for feasible and optimal research. The numbers of studies in relation to a specific analyte, methodologies used and in a specific diadgnostic area are scarce to derive the true potential of saliva as a diagnostic tool.

Ethics approval and consent to participate: Not applicable

Competing interests: Nil

Funding: Not applicable

Acknowledgements: Not applicable

References

[1]. Miles, Timothy S, Birgitte Nauntofte, and Peter Svensson. Clinical Oral Physiology. 1st ed. Copenhagen: Quintessence, 2004.

[2]. Kaczor-Urbanowicz, Karolina Elzbieta *et al*. "Saliva Diagnostics – Current Views and Directions". *Experimental Biology and Medicine* 2017; 242 (5): 459-472.

[3]. Wong, David T.W. "Salivaomics". *The Journal of the American Dental Association* 2012; 143: 19S-24S.

[4]. Mandel, Irwin D. The Diagnostic Uses of Saliva. *Journal of Oral Pathology and Medicine* 1990; 19(3): 119-125.

[5]. Iorgulescu, Gabriela. Saliva between Normal and Pathological. Important Factors in Determining Systemic and Oral Health. *Journal of Medicine and Life* 2009; 2(3): 303–307.

[6]. Company Profiles: Salimetrics LLC. 1st ed. Print.

[7]. Chai, Ryan C. *et al*. A Pilot Study To Compare The Detection Of HPV-16 Biomarkers In Salivary Oral Rinses With Tumour P16ink4a Expression In Head And Neck Squamous Cell Carcinoma Patients. *BMC Cancer* 2016; 16(1).

[8]. Yoon, Richard K., Arlene M. Smaldone, and Burton L. Edelstein. Early Childhood Caries Screening Tools. *The Journal of the American Dental Association* 2012; 143(7): 756-763.

[9]. Papaseit, Esther *et al*. Monitoring Nicotine Intake from E-Cigarettes: Measurement of Parent Drug and Metabolites in Oral Fluid and Plasma. *Clinical Chemistry and Laboratory Medicine* 2017; 55 (3).

[10]. Soares Nunes, Lazaro Alessandro, Sayeeda Mussavira, and Omana Sukumaran Bindhu. "Clinical and Diagnostic Utility of Saliva as a Non-Invasive Diagnostic Fluid: A Systematic Review". *Biochemia Medica* 2015; 25(2): 177-192.

[11]. Hansen AM, Garde AH, Persson R. Sources of biological and methodological variation in salivary cortisol and the- it impact on measurement among healthy adults: A review. *Scand J Clin Lab Invest* 2008; 68: 448-58.

[12]. Granger DA, Shirtcliff EA, Booth A, Kivlighan KT, Schwar- TZ EB. The "trouble" with salivary testosterone. *Psychone-uroendocrinology* 2004; 29: 1229-40.

[13]. Shirtcliff EA, Granger DA, Curran MJ. Use of salivary bio- markers in biobehavioral research: cotton-based sample collection methods can interfere with salivary immunoassay results. *Psychoneuroendocrinology* 2001; 26: 165-73. http://dx.doi.org/10.1016/S0306-4530(00)00042-1.

[14]. Carvalho, A. L. *et al*. Evaluation of Promoter Hypermethylation Detection in Body Fluids as a Screening/Diagnosis Tool for Head and Neck Squamous Cell Carcinoma. *Clinical Cancer Research* 2008; 14 (1): 97-107.

[15]. Nagler, Rafael *et al*. Heparanase Up-Regulation in Tongue Cancer. *Cancer* 2007; 110 (12): 2732-2739.

[16]. Petraityte, R. *et al*. Use of Saccharomyces Cerevisiae-Expressed Recombinant Nucleocapsid Protein to Detect Hantaan Virus-Specific Immunoglobulin G (IgG) and IgM in Oral Fluid. *Clinical and Vaccine Immunology* 2007; 14(12): 1603-1608.

[17]. Chakravarti, Anita, Monika Matlani, and Manisha Jain. Immunodiagnosis of Dengue Virus Infection Using Saliva. *Current Microbiology* 2007; 55 (6): 461-464.

[18]. Shpitzer, Thomas *et al*. A Comprehensive Salivary Analysis for Oral Cancer Diagnosis. *Journal of Cancer Research and Clinical Oncology* 2007; 133 (9): 613-617.

[19]. Giusti, Laura *et al*. Proteome Analysis of Whole Saliva: A New Tool for Rheumatic Diseases – The Example of Sjogren's syndrome. *Proteomics* 2007; 7 (10): 1634-1643.

[20]. Znaleziona, Joanna *et al*. Capillary Electrophoresis as a Verification Tool for Immunochemical Drug Screening. Biomedical Papers 2007; 151(1): 31-36.

[21]. Rauh, Manfred. Steroid Measurement with LC–MS/MS in Pediatric Endocrinology. Molecular and Cellular Endocrinology 301.1-2 (2009): 272-281.

[22]. Hu, S. *et al*. Salivary Proteomics for Oral Cancer Biomarker Discovery. *Clinical Cancer Research* 2008; 14 (19): 6246-6252.

[23]. Giovane, Alfonso, Antonio Balestrieri, and Claudio Napoli. New Insights Into Cardiovascular and Lipid Metabolomics. *Journal of Cellular Biochemistry* 2008; 105(3): 648-654.

[24]. Maternal Caffeine Intake during Pregnancy and Risk of Fetal Growth Restriction: A Large Prospective Observational Study. *BMJ* 2008; 337 (2): a2332-a2332.

[25]. Rai, Balwant *et al*. Adenosine Deaminase in Saliva as a Diagnostic Marker of Squamous Cell Carcinoma of Tongue. *Clinical Oral Investigations* 2010; 15 (3): 347-349.

[26]. Sashikumar, Radhika, and Ranganathan Kannan. Salivary Glucose Levels and Oral Candidal Carriage in Type II Diabetics. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology* 2010; 109(5): 706-711.