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**Dr. Uma Sudhakar**  
Professor and Head,  
Department of Periodontics,  
Thai Moogambigai Dental  
College and Hospital, Chennai,  
Tamil Nadu, India

**Dr. Snophia Suresh**  
Professor, Department of  
Periodontics, Thai Moogambigai  
Dental College and Hospital,  
Chennai, Tamil Nadu, India

**Arputha Madhalan C**  
Junior Resident, Thai  
Moogambigai Dental College and  
Hospital, Chennai, India

**Anu Priya B**  
Junior Resident, Thai  
Moogambigai Dental College and  
Hospital, Chennai, Tamil Nadu,  
India

**Prabakaran R**  
Junior Resident, Thai  
Moogambigai Dental College and  
Hospital, Chennai, Tamil Nadu,  
India

**Corresponding Author:**  
**Dr. Uma Sudhakar**  
Professor and Head,  
Department of Periodontics,  
Thai Moogambigai Dental  
College and Hospital, Chennai,  
Tamil Nadu, India

## Salivary diagnostics and its implementation in diagnosis of COVID19

**Dr. Uma Sudhakar, Dr. Snophia Suresh, Arputha Madhalan C, Anu Priya B and Prabakaran R**

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### Abstract

The coronavirus disease (COVID-19), epidemic started in Wuhan, caused by a novel coronavirus (SARS-CoV-2), has become a major public challenge around the world. Here, saliva diagnostic testing for COVID-19 is essential to controlling the global pandemic. The use of saliva has various advantages compared to collection of Nasopharyngeal swabs. The close contacts involved in swab collection have a risk to healthcare workers, and collection of saliva may reduce this risk. Presently, rapid testing is enchanting with the help of nasopharyngeal, oropharyngeal swab, bronchoalveolar lavage, sputum, urine, and blood. Saliva is demonstrating to be a capable non-invasive sample variety for the diagnosis of COVID-19, thus helping to detector the infection and prevent it from further spreading by prompt isolation. The saliva biomarkers have a potential to be an vital guide in COVID-19 diagnosis, making possible the development of sampling procedures. Salivary biomarkers connected with the improvement and evolution of COVID-19 could allow a well distinction among asymptomatic, mild, moderate or advanced disease.

**Keywords:** Saliva, infections, COVID-19, sampling, antibodies

### Introduction

The epidemic of coronavirus disease 2019 (COVID-19), instigated by a novel coronavirus (SARS-CoV-2), has become a major public health challenge around the world. In many cases, the rapid spread of infection transmission through person-to-person, either direct contact by sneeze, cough, or droplet inhalation, or contact transmission such as ocular contact or through mucous membranes of the eyes and nose and saliva, and the spread through droplets and aerosol particles [1].

Diagnostic testing for COVID-19 is essential to controlling the global pandemic. In spite of increase in diagnostic testing capacity for SARS-CoV-2, in many countries, testing is still inadequate to slow the COVID-19 pandemic. Many people still do not have access to COVID-19 tests, and some that do still experience long delays in receiving results due to imbalance between supply and demand at large testing centres. The use of saliva has various advantages compared to collection of Nasopharyngeal swab.

The close contacts involved in swab collection have a risk to healthcare workers, and collection of saliva may reduce this risk. Presently, rapid testing is taking place with the help of nasopharyngeal, oropharyngeal swab, bronchoalveolar lavage, sputum, urine, and blood [2]. All these approaches are invasive or uncomfortable to the infected person. It is observed that salivary glands are hosting SARS-COV-2 because of angiotensin-converting enzyme and the detection of high viral loads in the saliva and is playing a major role in virus spread, especially from individuals showing absolutely no symptoms. Further, saliva collection does not require specialised equipment, causes less patient discomfort, and may be a useful sample for self-collection.. Saliva is proving to be a promising non-invasive sample specimen for the diagnosis of COVID-19, thus helping to detector the infection and prevent it from further spreading by prompt isolation.

### Salivary Fluid as Possible Investigative Tool for Covid-19

Saliva is a distinctive body fluid secreted by the salivary glands. It has the purposes of lubricating oral mucosa, digesting food, cleaning and protecting the oral cavity, and is one of the most significant factors affecting homeostasis of the oral cavity. The major salivary glands parotid, submandibular and sublingual glands are the main sources of saliva exudation [3]

The development of the COVID-19 epidemic has emphasized the necessity for numerous diagnostic strategies to professionally estimate probable cases in order to deliver information on people exposure and immunity. These outfits presently include disease molecular analysis and quick host immune reaction assays. Saliva is a natural fluid in which SARS-CoV-2 can be established and for this purpose saliva has continued taken into consideration in the diagnosis of COVID-19 [4]. The investigative prospective of saliva was recognised by studies that shown that, like serum, saliva contains hormones, antibodies, growth factors, enzymes, microbes and their yields that can go into saliva through blood by passive diffusion, active transport or extracellular ultra-filtration. Hence, saliva can be a consistent liquid for observing the biological function of the body [2] While the little concentration of certain substances in saliva linked with the blood formerly revealed challenging, the creation of extremely complex molecular approaches and nanotechnology have to a great opportunity avoided this restriction.

### Saliva as a Possible Source for Spread of Virus

Human saliva is abundant of biologically active components, such as proline-rich proteins, mucins MG1 and MG2, and gp340. [6] These components intermingle with pathogens and cause multiple influences on their biological performance. The interface between viruses and saliva is a multifaceted biological process. Coronavirus is a group of enclosed single-stranded RNA viruses belonging to the order Nidovirales, the coronavirus family, and the coronavirus subfamily [5]. It has 26 known species and can be dispersed into four classes ( $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$ ). Merely the  $\alpha$  and  $\beta$  genus are human pathogenic strains. SARS-CoV, SARS-CoV-2 and the Middle East respiratory syndrome coronavirus (MERS-CoV) all belong to the  $\beta$  subdivision. Studies have revealed that early target cells for SARS-CoV infection comprise ACE2-positive cells/keratin epithelial cells in the salivary gland duct and other cells in the lungs, such as ACE2-positive cells/keratin alveolar epithelial cells, which recommended the salivary gland epithelial cells may be infected in vivo after entry of the virus (Liu *et al.*, 2011) [6]. Hence, the saliva produced by the infected salivary glands could be a significant source of virus, predominantly in early infection (Liu *et al.*, 2011). [6] At present, RT-PCR detection results of throat wash and saliva indicated that the content of SARS-CoV RNA in saliva was comparatively higher than that in throat wash, which maintained the possibility of oral droplet transmission of SARS-CoV (Wang *et al.*, 2004) [7]. The quantity, distance, and size of saliva droplets vary between individuals, signifying that the infectious intensity and transmission route of saliva droplets differ when the same pathogen is reduced. Each cough can produce about 3000 saliva droplets nuclei, which is almost equivalent to the quantity generated during a 5-min conversation. Each sneeze can create roughly 40,000 droplets of saliva covering several meters in the air. A regular exhalation can create saliva droplets that go beyond one meter in the air. Enormous saliva droplets with increased naturally

fall to the ground and small saliva droplets flutter by airflow like a cloud over longer distances. Henceforth, the virus has the likely to initiate disease through both short-distance and long-distance aerosol spread. There is increased risk of infection in people who have direct and unprotected contact with SARS patients (Tuan *et al.*, 2007) [8]. Therefore, dental clinicians in near contact with patients, salivary aerosols and plasma need to be highly endangered to condense the risk of infection, mostly during the epidemic period of COVID-19.

### Role of Saliva in Molecular Detection

The nature and clinical features linked with this virus are relatively distinctive including fever, non-productive cough mostly, malaise, dyspnoea and pneumonia. While sputum production, haemoptysis, headache and gastrointestinal symptoms such as diarrhoea, nausea and vomiting are less frequently presented symptoms [9]. Patients infected with COVID-19 show increased number of leukocytes, greater levels of plasma pro-inflammatory cytokines and abnormal respiratory findings. Swabs from the nasopharynx and oropharynx are the recommended upper respiratory tract specimen types for making diagnosis of COVID-19 the collection of swab requires close contact between healthcare workers and patients that not only increase the risk of transmission of the virus among healthcare workers but also causes discomfort resulting in bleeding, especially in condition like thrombocytopenia [9]. To overcome this problem, saliva has been found as an alternate source for making the diagnosis. It can be used for detecting respiratory viruses, including Coronaviruses because of high consistency > 90% with nasopharyngeal specimens.

### Salivary Fluid as Biomarkers for Covid-19 Diagnosis and Detection

Coronaviruses, such as SARS-CoV and Central East breathing syndrome (MERS)-CoV, have established methods to reduction or delay the production of interferon (IFN), triggering exuberant inflammatory responses leading to severe pulmonary conditions [10]. Salivary biomarkers and their role in point-of-care application have underlined the progress of the practice of more advanced technologies such as micro/Nano electro-mechanical systems, paper-based skill, fluorescent biosensors, photometric and electrochemical approaches, RNA-sequencing, liquid biopsy, electric field-induced release and measurement technique [11]. Markers of the inflammatory progression, such as cytokines and chemokine, can be measured in saliva. Such statistics has been recommended to be useful for the identification and prognosis of both oral cavity and systemic diseases. Hence, it is possible to create an inflammatory outline of COVID-19 by studying inflammation-related biomarkers in saliva. Interestingly, some of the known biomarkers in these studies such as C reactive protein, malic acid, guanosine monophosphate, lactate dehydrogenase, and proteins related with macrophage, platelet degranulation and supplement scheme pathways are visible to be present in saliva [12]. These results support the possible use of saliva-based metabolic/protein/lipid biomarkers as a non-invasive approach for patient stratification in COVID-19 disease. Metabolomics is a method used in the study of small molecules from the metabolic profile of cells, tissues or fluids, which help in the classification of a phenotype. These molecules termed biomarkers, are essential in clinical practice for defining the state of a disease [13]. Thus, metabolomics has helped to recognise biomarkers with investigative potential

and explanation of metabolic pathways in the most diverse clinical situations, including those containing viral and bacterial pathogens, and more exactly viruses that cause respiratory diseases such as influenza and SARS. Patients recovered from severe acute respiratory syndrome caused by SARS-CoV were recruited after 12 years of infection for metabolic evaluation of the consequences of the disease [14].

The assessment of patients' serum with healthy persons showed differences in organic acids, amino acids, phospholipids, carnitine and inositol derivatives. These results represent the practical application of metabolomics in the estimation of long-term effects. MicroRNAs, non-coding RNAs of 20-nucleotide to 22-nucleotide length, silencing gene appearance by a transcript-specific target-mediated inhibitory action, play a key role in numerous cellular processes including cell development and distinction immunity, cell metabolism, proliferation, apoptosis and cancer [15]. The importance of monitoring microRNA is associated to the fact that a single microRNA can be occupied in several cellular regulatory pathways, which involve dissimilar molecules. There are studies reporting a specific microRNA upregulation and down regulation of nuclear factor- $\kappa$ B pathway and IFN pathway associated with some viruses including respiratory virus infection [16]. Moreover, in this context, since microRNAs related with extracellular vesicles are recognized to be protected from enzymatic degradation, several studies have been focused on the investigation of the expression of microRNAs in extracellular vesicles gained from saliva as potential biomarkers. Therefore, the fact that microRNA existing in biological fluid can replicate the molecular event within the cellular background, make them a potential exhaustive marker to check the cell-infection status; this is predominantly important in a low replicative condition in which virus cannot be present in biological fluid [17] provides an opportunity to evaluate virus pathological effect-associated diseases as in COVID-19.

### Salivary Antibodies against Covid-19

Viral antibodies have been spotted in saliva and the immunisation status of measles, rubella, mumps and hepatitis can be tested by analysing IgG, IgM and IgA in oral fluids [18]. In addition to RT-PCR-based RNA detection of SARS-CoV-2, initially studies have been reported, capable results for the finding of IgM and IgG against SARS-CoV-2 in serum/plasma samples of patients with SARS-CoV-2. [19] Regarding SARS-CoV-2, only a study procedure aimed to analyse IgG, IgM and IgA in changed biological fluids including self-collected saliva for rapid SARS-CoV-2 diagnosis has been published [20]. Viral antibodies have been noticed in saliva and the immunisation status of measles, rubella, mumps and hepatitis can be confirmed by analysing IgG, IgM and IgA in oral fluids [18]. Though, there are so far no outcomes describing the presence of antibodies against SARS-CoV-2 in human saliva. This clearly permits future studies on the potential use of salivary immunoglobulins for COVID-19 in diagnostics, disease progression and immunisation monitoring.

### Methods of Salivary Testing For Sars-Cov-2

Salivaomics is the study of salivary "omics" methodologies including the genome, the epigenome, the transcriptome, the proteome, the microbiome, and the metabolome [21]. The ability to assemble a model in a non-invasive, safe, and total effective method, with the profits of advanced patient ease

and compliance makes the adoption of saliva for each of these "omics" techniques an attractive proposition for all parties concerned (patients, researchers, and clinicians) here are many properties of human saliva that attract clinicians or researchers to adopt the use of saliva specimens and reinforce the use of this non-invasive fluid in diagnostic algorithms [22]. Some of these are highlighted below: Non-invasive, Simple collection protocols, Non-infectious sample, Easily disposal, Effortlessly portable, economic, Not subject to cultural and religious, Safe and effective, Advanced patient acquiescence.

The actual principal occurrence of a method for saliva assemblage from a patient was in the initial 19th period (1934) by Wainwright for the investigation of salivary calcium (Ca<sup>2+</sup>). In Wainwright's method, the patient's head was angled forward with the opening indicating vertically downwards and saliva was permitted to drip from the mouth into a filter funnel [23]. Standard healthy adults yield around 0.5 to 1.5 litres of saliva per day (or nearly 0.5 mL/min) but in various systemic diseases, and in pathological and biological conditions, there may be a considerable (negative) impact on the salivary flow rate. [24] [Table 1]

Two companies from the United States, namely Epitope, Inc, were two of the early pioneers in the area; each developed commercially viable saliva collection devices in the 1990s, and each of these has been used in proteomic analysis and other areas of research and clinical practice [31]. The Ora Sure Device from Epitope/Ora Sure was the first saliva collection device to be linked to a clinical test for the human immunodeficiency virus (HIV) and the company was successful in gaining Food and Drug Administration (FDA) approval for the device in conjunction with a laboratory enzyme-linked immunosorbent assay (ELISA) test for the HIV virus. Saliva [10]. These early procedures prompted new improvements in saliva collection method equipment instigating in devices that yield "domestic" specimens, thus permitting clinicians to analyse saliva more easily than before. Other devices that have been used historically include the Salivate device from S Salimetrics Oral Swab (SOS). Fresher devices on the marketplace "parodist" whole saliva pool using passive drool. These devices, which includes the (A) Salivette® (Sarstedt); (B) Quantisal® (Immunoanalysis); (C) SCS® (Greiner-BioOne), Super•SAL™ (Figure D) and Versi•SAL® (Figure E) technologies. These devices (Figure D,E) have been used successfully to collect hormones [31], proteins, and biomarkers potentially useful in the diagnosis of Parkinson's disease [31] as well as infectious agents including the Ebola virus and Lassa fever. The serious entity is that the modern group of strategies provide a standardized trial of saliva, characteristic of complete mouth saliva.

### PCR Detection in Sars-Cov-2 with Salivary Fluid Testing For Sars-Cov2

At Present, RT-PCR is the most universally used diagnostic test for the exposure of SARS-CoV-2 RNA in the biological samples. For comprehensive testing as in the case of SARS-CoV-2, accurate collection of the type and the site of biological specimen collection is crucial for obtaining unflinching test results [32]. Biological samples from the upper tract such as nasopharyngeal swabs, oropharyngeal swabs, throat swabs, nasal swabs and lower tract such as bronchoalveolar lavage respiratory tract scan and tracheal aspirates can be used for the detection of SARS-CoV-2 with varying degree of test sensitivity [33] Presently, nasopharyngeal/oropharyngeal swabs where virus samples are collected by separately rubbing the nasopharyngeal wall and

the posterior pharynx/tonsillar areas through mini tip swabs, are regularly used for SARS-CoV-2 detection. Despite the extensive use, the collection of nasopharyngeal/oropharyngeal swabs has a number of restrictions. The assembly of these washes is less reasonable to patients as associated with non-invasive approaches like saliva collection, as it tends to cause patient discomfort and even bleeding. Likewise, the risk for disease transmission to the healthcare workers when collecting these samples is high, as it needs active participation of the test taker. Also collection of these samples stresses the use of personal protective and healthcare resources, both of which tend to be in little source in a pandemic like COVID-19 [34].

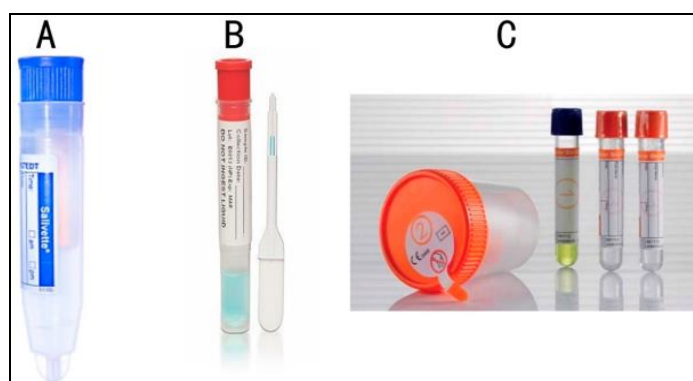
**Advantages and Disadvantages of Salivary Sampling**

Saliva has been studied comprehensively as a possible diagnostic tool and it is likely to become a auxiliary for other biological fluids such as serum or urine in disease analysis [18]. Compared with other investigative fluids, saliva sampling has both advantages and disadvantage in use for the diagnosis of

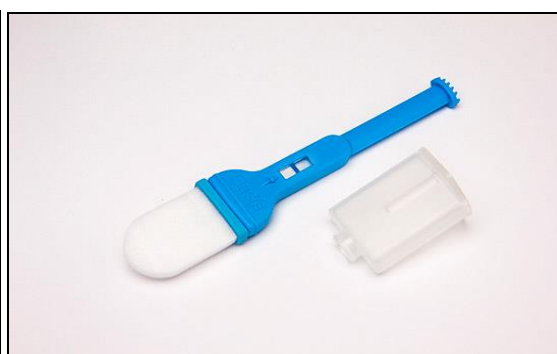
COVID-19. (Table 2)

**Future of Salivary Sampling in SARS-COV-2**

Saliva collection is relatively stress-free for patients as well as being easy, inexpensive, and non-invasive with minimal equipment required. It should also minimize the nosocomial transmission of COVID-19 to healthcare workers. The usage of saliva-based SARS-CoV-2 testing deals several clinical benefits and is scientifically well created. Saliva-based testing can be an substitute to the more extensively used nasopharyngeal and oropharyngeal washes for COVID-19 diagnosis and disease monitoring. The beneficial role of saliva as a quick, non-invasive diagnostic modality and the various possibilities it presents with, for investigation, during the course of the disease process, prognosis or presence of any antibodies to the novel COVID-19 virus, needs further exploration. Additionally, the involvement of any other receptors or cellular proteases which may throw more light on the pandemic disease pathogenesis may pave way to targeted drug therapies.



Versi SAL®, (E)



Super SAL by Oasis Diagnostics® Corporation

**Fig A:** (A) Salivette® (Sarstedt); (B) Quantisal® (Immunalysis); (C) SCS® (Greiner-BioOne) [9, 10]

**Table 1:** Table represents the group of mouth liquid and collection method of salivary sampling

Group of Complete Mouth Liquid	Method of Collection and Type of Collection Device
Whole Saliva (WS)	Patients should refrain from eating, drinking, and oral hygiene actions for at minimum 1 h before saliva collection. (Optimal collection time is 8–10 a.m.). Once collection implements a 1 min oral rinse by distilled water and then after 5 min collects ~5 mL of saliva. Collected sample must be processed in the laboratory within 1 h. [25]
Un-stimulated Full Saliva	Passive drooling: In this method limit oral movement and drain saliva from the lower lip into a plastic vial. Spitting technique: Teach subject to spit into a collection vial. In this method 14 times more bacterial contamination is introduced into the sample [26]
Stimulated Whole Saliva	For the stimulation of glands, chewing unlike things like natural gum, a piece of paraffin wax, citric acids, and powdered drink crystals have been used. [27]
Parotid Gland	Method introduced by Carlson and Crittenden (1910). In this process a dual chambered copper beaker with two opening tubes is used. One termination grasps the cup in place using vacuum suction. The following half acts as a gathering vehicle for saliva. Sample collection can be boosted by spreading citric acid (10%; 1 mL) on the dorsum of tongue every 30 s. Remove the first 1.5 mL of saliva prior to trial collection. [28]
Submandibular and Sublingual Gland	Truelove, Bixler, and Merrit (1967) castoff a “V”-shaped accumulator. This method is similar to that for parotid gland collection, but in this case the initial 2 mL is discarded [29].

Minor Glands	Kutscher <i>et al.</i> (1967) used capillary tubes for accumulating saliva from minor glands situated at the everted surface of the lower lips [30].
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**Table 2:** Table represents the most common advantages and disadvantages of saliva sampling method in diagnosis of covid19

Advantages	Disadvantages
Safer collection for health professionals than other biological trials such as nasopharyngeal swabs and blood. Non-invasive method for diagnosis of the disease	Not at all times reliable for measurement of certain markers
No patient discomfort and anxiety for sampling.	Substances of saliva can be predisposed by the method of collection, degree of stimulation of salivary flow, inter individual dissimilarity and oral hygiene status.
Easy collection and applicable in isolated areas.	Serum indicators can reach whole saliva in an unpredictable way.
Comparatively cheap technology. Economical	Medications may upset salivary gland function and subsequently the quantity and composition of saliva.
Suitable for children, anxious, disabled and elderly patients.	Possibility for degradation of salivary proteins due to occurrence of proteolytic enzymes.
Potential multisampling.	
Easy to handle, No need for expensive equipment or instruments Simply needs a sterile container	

### Conclusion

The examination for salivary biomarkers linked with the improvement and evolution of COVID-19 could allow a better distinction between asymptomatic, mild, moderate or advanced disease. Saliva biomarkers have a prospective to be an essential guide in COVID-19 prognosis, making possible the development of sampling procedures. Knowledge of this kind might lead to the development of point-of-care devices, which can be extremely useful for understanding of the evolution of contagions and immunological responses in population studies. Right now, in this uncontrollable pandemic situation, all research centers, health agencies, and health care providers must explore the diagnostics opportunity and rapidly develop automated molecular point-of-care assays. This write-up will help epidemiologists, virologists, and clinicians to understand the importance of saliva in diagnostic testing as well as the transmission of the disease.

### References

- Xu H, Zhong L, Deng J, Peng J, Dan H, Zeng X *et al.* High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. *Int J Oral Sci* 2020;12(1):8
- Malamud D. Saliva as a diagnostic fluid. *Dent Clin North Am* 2011;55:159-78
- Sapkota D, *et al.* *J Clin Pathol* 2020;0:1-6. doi:10.1136/jclinpath-2020-206834 COVID-19 salivary signature: diagnostic and research opportunities
- Zhou P, Yang X-L, Wang X-G, *et al.* A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 2020;579:270-3.
- de Almeida Pdel V, Gregio AM, Machado MA, de Lima AA, Azevedo LR. Saliva composition and functions: A comprehensive review. *The Journal of Contemporary Dental Practice* 2008;9(3):72-80. <https://doi.org/10.5005/jcdp-9-3-72>
- Liu L, Wei Q, Alvarez X, Wang H, Du Y, Zhu H *et al.* Epithelial cells lining salivary gland ducts are early target cells of severe acute respiratory syndrome coronavirus infection in the upper respiratory tracts of rhesus macaques. *Journal of Virology* 2011;85(8):4025-4030. <https://doi.org/10.1128/Jvi.02292-10>
- Wang W-K, Chen S-Y, Liu I-J, Chen Y-C, Chen H-L, Yang C-F *et al.* Detection of SARS-associated coronavirus in throat wash and saliva in early diagnosis. *Emerging Infectious Diseases* 2004;10(7):1213-1219.
- Tuan PA, Horby P, Dinh PN, Mai LTQ, Zambon M, Shah J *et al.* SARS transmission in Vietnam outside of the healthcare setting. *Epidemiology and Infection* 2007; 135(3):392-401.
- Role of Saliva as a Viable Bio Sample for COVID-19 Detection Rabia Anjum
- Fehr AR, Channappanavar R, Perlman S. Middle East respiratory syndrome: emergence of a pathogenic human coronavirus. *Annu Rev Med* 2017;68:387-99.
- Kaczor-Urbanowicz KE, Martin Carreras-Presas C, Aro K *et al.* Saliva diagnostics - Current views and directions. *Exp Biol Med* 2017;242:459-72.
- Galhardo LF, Ruivo GF, de Oliveira LD, *et al.* Inflammatory markers in saliva for diagnosis of sepsis of hospitalizes patients. *Eur J Clin Invest* 2020;50:e13219.
- Goldansaz SA, Guo AC, Sajed T *et al.* Livestock metabolomics and the livestock metabolome: a systematic review. *PLoS One* 2017;12:e0177675.
- Wu Q, Zhou L, Sun X, *et al.* Altered lipid metabolism in recovered SARS patients twelve years after infection. *Sci Rep* 2017;7:9110.
- O'Brien J, Hayder H, Zayed Y *et al.* Overview of microRNA biogenesis, mechanisms of actions, and circulation. *Front Endocrinol* 2018;9:402.
- Leon-Icaza SA, Zeng M, Rosas-Taraco AG. microRNAs in viral acute respiratory infections: immune regulation, biomarkers, therapy, and vaccines. *ExRNA* 2019, 1.
- Martelli F, Mencarini J, Rocca A, *et al.* Polyomavirus microRNA in saliva reveals persistent infectious status in the oral cavity. *Virus Res* 2018;249:1-7.
- Cruz HM, de Paula VS, da Silva EF, *et al.* Utility of oral fluid samples for hepatitis B antibody detection in real life conditions. *BMC Infect Dis* 2019;19:632.
- Zhao J, Yuan Q, Wang H, *et al.* Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. *Clin Infect Dis* 2020. doi:10.1093/cid/ciaa344. [Epub ahead of print: 28 Mar 2020].
- Sullivan PS, Sailey C, Guest JL, *et al.* Detection of SARS-CoV-2 RNA and antibodies in diverse samples: protocol to validate the sufficiency of Provider-Observed, Home-Collected blood, saliva, and oropharyngeal samples. *JMIR Public Health Surveill* 2020;6:e19054.
- Ai J, Smith B, Wong DT. Saliva ontology: An ontology-based framework for a Salivaomics Knowledge Base. *BMC Bioinform.* 2010, 11.
- Besnard M, Lastère S, Teissier A, Cao-Lormeau V,

- Musso D. Evidence of perinatal transmission of Zika virus, French Polynesia, December 2013 and February 2014. *Euro surveillance* 2014, 19.
23. Wainwright WW. Human saliva II. A technical procedure for calcium analysis. *J. Dent. Res* 1934;14:425-434.
  24. Chiappin S, Antonelli G, Gatti R, de Palo EF. Saliva specimen: A new laboratory tool for diagnostic and basic investigation. *Clin. Chim. Acta* 2007;383:30-40.
  25. Henson BS, Wong DT. Collection, storage, and processing of saliva samples for downstream molecular applications. *Methods Mol. Biol* 2010;666:21-30.
  26. Michishige F, Kanno K, Yoshinaga S, Hinode D, Takehisa Y, Yasuoka S. Effect of saliva collection method on the concentration of protein components in saliva. *J. Med. Investig* 2006;53:140-146.
  27. Gröschl M, Rauh M. Influence of commercial collection devices for saliva on the reliability of salivary steroids analysis. *Steroids* 2006;71:1097-1100.
  28. Carlson AJ, Crittenden AL. The relation of ptyalin concentration to the diet and to the rate of salivary secretion. *Exp. Biol. Med.* 1909;7:52-54.
  29. Truelove EL, Bixler D, Merritt AD. Simplified method for collection of pure submandibular saliva in large volumes. *J. Dent. Res.* 1967;46:1400-1403.
  30. Kutscher AH, Mandel ID, Zegarelli EV, Denning C, Eriv A, Ruiz L *et al.* A technique for collecting the secretion of minor salivary glands: I. Use of capillary tubes. *J. Oral Ther. Pharmacol.* 1967;3:391-392.
  31. Human Saliva Collection Devices for Proteomics: An Update by Zohaib Khurshid 1OrcID, Sana Zohaib 2OrcID, Shariq Najeem 3OrcID, Muhammad Sohail Zafar 4,\*OrcID, Paul D. Slowey 5 and Khalid Almas 6 Published, 2016
  32. Xie C, Jiang L, Huang G *et al.* Comparison of different samples for 2019 novel coronavirus detection by nucleic acid amplification tests. *Int J Infect Dis* 2020;93:264-7.
  33. Lin C, Xiang J, Yan M, *et al.* Comparison of throat swabs and sputum specimens for viral nucleic acid detection in 52 cases of novel coronavirus (SARS-Cov- 2)-infected pneumonia (COVID-19). *Clin Chem Lab Med* 2020;58:1089-94.
  34. Sapkota D, Thapa SB, Hasséus B, *et al.* Saliva testing for COVID-19? *Br Dent J* 2020;228:658-9.
  35. Zhang C-Z, Cheng X-Q, Li J-Y, *et al.* Saliva in the diagnosis of diseases. *Int J Oral Sci* 2016;8:133-7.