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Review Article

Saliva: A double-edged sword

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Abstract

The COVID-19 virus is primarily known to affect the respiratory system of infected individuals. The mode of transmission is predominantly said to be through droplet spread, and this poses a great threat to the dental profession, as most dental treatments are aerosol-generating procedures (AGPs). The virulence of the COVID-19 virus initiates an antiviral response after the viral genome is released into the human cell cytoplasm. ACE2 receptors are predominantly seen in the salivary gland and tongue tissues. The saliva collected directly from the salivary glands had shown nucleic acid of the COVID-19 virus. Most dental treatments involve exposure to saliva aerosols, blood, and sharp instruments. Hence, it is important to understand the role of saliva in the transmission of COVID-19 virus. This review highlights the physiobiological aspects of Saliva, diagnostic tests available, and recommendations for dentists to facilitate dental treatment with a low risk of disease transmission.

Keywords: Saliva, COVID-19, Infection; Infection control, Nasopharyngeal swab; Oropharyngeal swab.

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1. Introduction

The emerging COVID-19 pandemic has been declared a public health emergency of international concern (PHEIC) by the World Health Organization (WHO), based on the International Health Regulations.[1] The COVID-19 virus is primarily known to affect the respiratory system of individuals who are infected. The incubation period is said to be from 1 – 14 days, and 3-7 days is the most infectious period.[2] The mode of transmission is predominantly attributed to droplet spread, posing a significant threat to the dental profession, as most dental treatments are aerosol-generating procedures (AGPs). The existing literature provides evidence that the COVID-19 virus mainly attaches to human cells that contain the angiotensin-converting enzyme 2 (ACE2) receptor through the glycoprotein on its surface.[2] The virulence of the COVID-19 virus initiates an antiviral response after the viral genome is released into the human cell cytoplasm. ACE2 receptors are predominantly seen in the salivary gland and tongue tissues.[1] The saliva collected directly from the salivary glands had shown nucleic acid of the COVID-19 virus.[3] Most dental treatments involve exposure to saliva aerosols, blood, and sharp instruments. Hence, it is important to understand the role of saliva in the transmission of COVID-19 and also as a diagnostic tool to detect the COVID-19 virus load. This review highlights the physiobiological aspects and functional role of saliva, a diagnostic tool for early detection of diseases/disorders, and recommendations for dentists to facilitate dental treatment with a low risk of disease transmission.

2. Discussion

2.1. Physiobiological aspects of saliva

Human saliva is composed of 99% water, 0.3% proteins, and 0.2% inorganic substances and organic molecules. The organic component is composed of enzymes such as amylases, peroxidase, lipase, lysozyme, lactoferrins, kallikreins, cystatins, hormones, and growth factors. The inorganic component is composed of sodium, potassium, calcium, magnesium, chloride, and carbonates.[4]

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There are three major salivary glands—the submandibular (about 65%), parotid (about 20%), and sublingual (about 5–7%)—that secrete around 0.5 -1.5L of saliva. The nervous system of the salivary glands includes both sympathetic and parasympathetic neural systems. The sympathetic nervous system increases the volume of the saliva compared to the parasympathetic nervous system.[5]

Human saliva is primarily composed of water and organic molecules, including proteins, enzymes, salts, and immunoglobulins. The primary functions of saliva include lubricating the oral mucosa, aiding in food digestion, and protecting against pathogens. It plays a crucial role in maintaining the homeostasis of the oral cavity and serves as a key marker in many systemic and oral diseases. Owing to its anti-bacterial/ antiviral action through the enzymes and proteins (mucin, glycoprotein, statherin, histatin, lysozyme, peroxidase, salivary agglutinin, sIgA, SLPI, and α, β defensins), saliva is called as Gatekeeper of the oral cavity.[4]

2.2. Functions of saliva[5]

Defense function: Saliva acts as a defense mechanism due to its components, including myeloperoxidase, lactoferrin, salivary peroxidase, and lysozyme.

Lubricant function: Saliva lubricates the hard and soft oral tissues and acts as a biomarker for systemic diseases. The proteins in saliva form a biofilm on the tooth surfaces.

Digestive function: The enzymes in saliva play a key role in the digestion of food. Saliva dilutes the food, converts it to simple carbohydrates, and aids in clearing the food. The buffering action of saliva aids in maintaining the pH 6.5 – 7.4 of the oral cavity and ensures the prevention of caries.

Taste function: Saliva transports the tastant from the mouth to the taste buds. The tastant diffuses through the saliva and approaches the taste buds.

2.3. Applications of saliva as a diagnostic tool[6]

Saliva is considered functionally equivalent to human serum owing to its organic and inorganic components. Many of the components enter saliva via passive diffusion, and hence saliva reflects the biological functioning of the body.

Dental caries: An increased count of Streptococcus mutans and Lactobacillus in saliva indicates a higher risk of dental caries. Apart from this, decreased levels of proline-rich proteins, histatins, and statherin are also associated with dental caries.

Periodontal diseases: A rise in salivary immunoglobulins IgA, IgM, and IgG indicates a risk of periodontitis. A decreased level of lysozyme, histatin, is considered a risk factor for periodontal diseases. Numerous other biomarkers act as key indicators of periodontal disease.

Oncology: A wide variety of tumour markers are present in saliva that could be used to identify cancers (Oral, Pancreatic, Gastric, Lung). Furthermore, the genetic analysis of the tumor biomarkers is a valuable aid for the diagnosis of malignancies.

Hormone levels: Saliva is a key to analyzing the hormonal levels of testosterone, aldosterone, progesterone, and estradiol.

Iron deficiency anemia: Ferritin levels in saliva are a useful guide to observe anemia.

Cardiac diseases: A key indicator of acute myocardial infarction, cardiovascular diseases

Autoimmune disorders: Sjogren's syndrome has a reduced quantity of salivary secretion.

Autoimmune skin diseases: A key indicator of psoriasis, bullous pemphigoid (BP), pemphigus vulgaris (PV).

Wound healing: The growth factors (fibroblast growth factor, insulin growth factor, and nerve growth factor), and clotting factors (IXa, VIII, and XI) aid in wound healing.

Infections: Helicobacter Pylori, measles, Rubella, Hepatitis A, B, and C, viral infections such as rhinovirus, enterovirus, adenovirus, bocavirus, and COVID-19 virus. The DNA analysis of saliva is a useful tool to understand oral bacteria profiling and has further applications in forensics. Levels of IgG antibody play a key role in the screening of bacterial and viral infections.

Endocrine disorders: Diabetes mellitus, adrenal and testicular tumors, hirsutism, menstrual problems.

Neurological disorders: Psychiatric disorders, schizophrenia, depression, stress-related disorders.

Gastrointestinal diseases: Inflammatory bowel disease (IBD), Ulcerative colitis.

2.4. Role of saliva as a diagnostic tool for COVID-19 virus

The cells of the salivary glands, ductal tissues, and tongue tissue predominantly possess the ACE2 receptor, which hence the attachment of the COVID-19 Virus to human cells occurs. Other tissues that express the ACE2 receptor include the lungs, gallbladder, esophagus, colon, ileum, and liver.[1] The expression of the Furin enzyme cleaves the COVID-19 virus envelope, increasing the concentration on the tongue. Hence, the oral cavity is said to halt a significant load of the virus due to the presence of the ACE2 receptor and the furin enzyme on the tongue, as well as the ACE2 receptors in the salivary glands. Apart from the saliva droplets that can be released during dental treatment, around 3000 and 40,000 saliva droplets are generated in one cough and sneeze.[5,6] Saliva can be used as a diagnostic tool, particularly for mass screening in large populations. Chairside screening of COVID-19 with saliva is a valuable aid for dentists.[7] A

scoping review reported that the viral load in salivary samples was higher compared to that in nasopharyngeal swabs.[8] A systematic review concluded that the saliva test is a less costly alternative to the nasopharyngeal swab test.[9] However, another systematic review comparing saliva, NP, and nasal swabs concluded that the NP swab test is the gold standard for COVID-19 detection.[10]

The **Table 1** illustrates studies that showed the COVID-19 Virus isolated from the saliva and the importance of saliva as a diagnostic tool.

The COVID-19 virus can bind to ACE-2 receptors on the epithelium of salivary tissues, interact with them, and imitate and lyse cells to trigger clear signs and symptoms, such as discomfort, inflammation, and pain in major salivary glands. After the cytolytic movement of the COVID-19 virus lyses the acinar cells, salivary amylase is released into the blood. The discharged cytokines encourage the inflammatory response that destroys the tissue of the salivary organs as the immunopathological process proceeds. After the serious stage, the viral load increases in salivary glands, which may initiate chronic sialadenitis.[24]

The organic components of saliva are used as biomarkers for the identification of disorders related to endocrine, autoimmune, genetic, and infectious diseases. Saliva sampling can improve SARS-CoV-2 diagnostic techniques. Saliva samples are easier to collect than nasal and throat samples; the technique is noninvasive, presumably preferred by the participant, and does not require sampling proficiency. Additionally, saliva sampling eliminates the need for swabs and transport media, which have been in limited supply during the pandemic.[1]

Table 1:

Author	Specimen	Percentage
To et al, 2020[11]	Nasopharyngeal (NPS) and bronchopulmonary secretions from deep throat	Out of 23 COVID-19 patients included in this study, 20 cases of COVID-19 were isolated from the secretions from the throat and Nasopharynx
Zhang et al, 2020[12]	Saliva, throat swabs	50% of subjects enrolled in the study had the COVID-19 virus in saliva collected from throat swabs
Williams et al, 2020[13]	Nasopharyngeal specimens (NPS)	39 out of 62 nasopharyngeal swabs tested positive; out of these 39 samples, 33 salivary samples tested positive for the COVID-19 virus
Azzi et al, 2020[14]	Drooling Saliva	All the salivary specimens tested 100% positive for the COVID-19 virus
Justo et al, 2021[15]	Saliva and NPS	Saliva specimens presented a sensitivity of 98.6% compared to nasopharyngeal swabs.
Sakanashi et al, 2021[16]	Saliva and NPS	A total of 19 Salivary specimens and 15 nasopharyngeal swab specimens showed positive results, respectively.
Hitzenbichler et al, 2021[17]	Throat washings (TW), nasopharyngeal swabs (NPS), and oropharyngeal swabs (OS)	The study concluded that throat washings (TW), and oropharyngeal swabs (OS) can be considered for SARS-CoV-2 diagnostics
Jamal et al, 2021[18]	Nasopharyngeal swabs (NPS) and saliva	Study findings included, sensitivity was 89% for nasopharyngeal swabs and 72% for saliva
Tutuncu et al, 2021[19]	NPS and Saliva	Among 53 patients with potential SARS-CoV-2 infection, the real-time RT-PCR was positive in the saliva specimens in 48 (90.56%) patients.
Landry et al, 2020[20]	NPS, Saliva	Real-time RT-PCR of pure saliva had an overall sensitivity for SARS-CoV-2 RNA detection of 85.7% when compared to simultaneously collected NPS.
Canette et al, 2021[21]	NPS and saliva	The detection rate of SARS-CoV-2 in saliva was similar to that for nasopharyngeal swabs.
Güçlü et al 2021[22]	NPS and saliva	SARS-CoV-2 was detected in 27 (42.2%) patients' saliva samples. While the sensitivity and positive predictive value of saliva samples were 85.2%

3. Advantages of Saliva as a Diagnostic Tool

1. Cost-effective
2. Saliva doesn't clot and hence requires less manipulation
3. Non-invasive methods as a needle for sample collection, aren't required
4. High Sensitivity and specificity of the test
5. Home testing option available with advanced assays
6. Minimal requirement of equipment and training to collect saliva

4. Recommendations

Most of the dental procedures involve aerosol generation. Especially, aerosols generated from high-speed handpieces and ultrasonic cleaning devices pose a significant threat to dentists, auxiliaries, and other patients in the dental operatory. The aerosol from an infected person is said to reach about 2m in radius in the dental operatory.[26] Hence, there is a need to follow the strict guidelines and recommendations as follows:

1. Prioritize the treatments: All efforts should be made to provide remote consultation through Telemedicine and teledentistry. The 3A's 'advice, analgesia, and antimicrobials' are recommended as an effective strategy to deal with most of the dental emergencies. The essential and non-essential procedures in various dental specialties as shown in **Figure 1**. This figure gives a quick guidance checklist to prioritize the treatments, and the essential procedures that require an office visit are discussed below.[27] Every effort should be made to assess the situation before developing a treatment plan.

    a. Restorative procedures that involve moderate/severe decay, painful tooth fractures, and placement of crowns following root canal treatment.
    b. Endodontic procedures for infections, swelling, and acute pain.
    c. Surgical procedures that include extractions for infections, swelling, and 3rd molar extraction.
    d. Ortho procedures for bracket/wire fractures.
    e. Periodontic procedures that include risk factors following a therapy.
    f. Prosthodontic procedures that involve dentures.

2. Adequate ventilation of operatory: The dental operatory room and waiting room should have adequate ventilation. The aerosol can be viable in air up to 3 hours in aerosol and 5.6 hours on stainless steel, and 6.8 hours on plastic surfaces.[28] Hence, strict sterilization and disinfection guidelines should be followed to prevent the transmission of aerosols to the patients, dental practitioners, and office staff.
3. Necessary equipment: When the in-office consultation is inevitable, the recommendation is that dentists and auxiliaries must follow the infection control procedures proposed by health authorities.[29] The personal protection equipment (PPE) includes Gloves, masks (Surgical, N95, FFP3 respirators), gowns, face shields, and eye protection (goggles) as seen in **Figure 2**. Occupational Safety and Health Administration (OSHA)[30] has proposed powered air-purifying respirators (PAPRs) or supplied air respirators (SARs). The dental staff have to be adequately trained in donning and doffing of PPE as it is a critical step in infection control. The steps are illustrated in **Figure 3** and 4. The pre-donning instructions before initiating the dental procedure and doffing of the personal protective equipment (PPE) post-treatment must be followed as strictly as possible. During the procedure, if any damage occurs to the PPE, immediate action should be taken to replace it before proceeding with treatment.
4. Disinfection intra-operative: A quick pre-procedure mouth rinse with 0.2% povidone iodine mouthwash/ 0.5-1% hydrogen peroxide,[31] 0.12%-0.2% chlorhexidine gluconate[32] significantly reduces the viral load. This important additional step ensures, prevention/ reduction of the transmission of the aerosols.
5. Avoid aerosol generation: These include avoiding intraoral radiographs and preferring extraoral radiographs whenever possible, prioritizing treatment with hand instruments (minimally invasive procedure) using rubber dam isolation and high-volume suction devices. Limiting treatments to essential procedures as applicable.

SPECIALTY	PROCEDURE TYPE	ESSENTIAL	NON-ESSENTIAL
Restorative	• **Fillings/Restorations**		
	Mild decay		X
	Moderate decay	X	
	Severe Decay	X	
	• **Fracture tooth repair**		
	Pain or uncomfortable	X	
	No pain or discomfort		X
	• **Crown**		
	For completion of care for moderate to severe decay or to complete RCT	X	
	Proactive replacement of restoration without decay		X
	• **Veneers**		X
Cosmetics	• **Cosmetic procedures**		X
Endodontics	Active infection	X	
	Patient in pain	X	
	Swelling/cellulitis	X	
Emergencies	Any patient with urgent needs	X	
Hygiene	New Patient		X
	Recall		X
	Continuing care		X
Oral Surgery	• **Extractions**		
	Active infection	X	
	Patient pain	X	
	Swelling/cellulitis	X	
	Asymptomatic 3rd molars		X
	• **Implants**		X
Orthodontics	New bandings		X
	Wire or bracket fractures	X	
	Recall		X
	Debond*		X
	*Orthodontist to make judgement on recall time		
Periodontics	• **Initial therapy, debridement or maintenance**		
	Contributory risk factors	X	
	No risk factors		X
Prosthodontics	• **Bridges**		X
	• **Dentures**	X	
Paediatrics	Adult guidelines to be followed		

Figure 1: Essential and non-essential procedures in various dental specialties.[7]

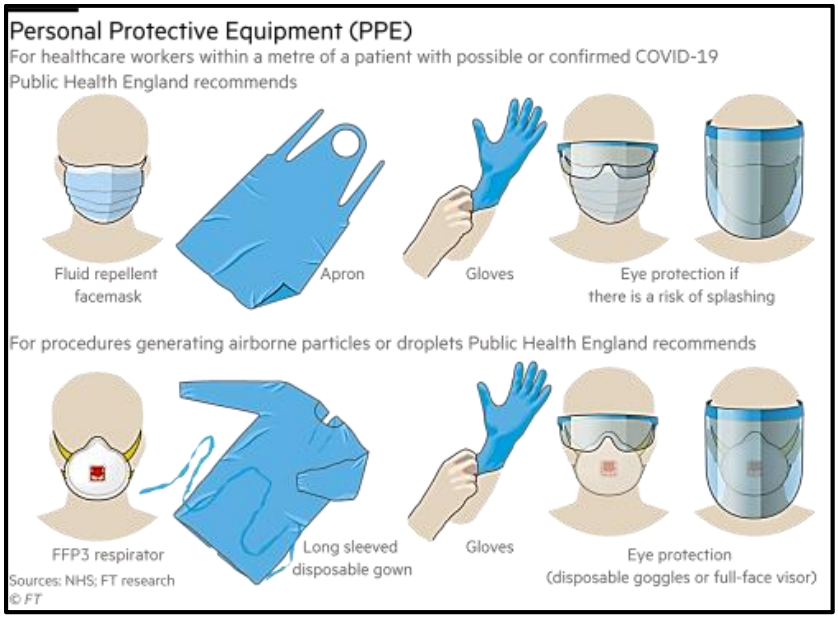

Figure 2: Public Health England recommendations for personal protective equipment use. (https://www.ft.com/content/18bf272b-0b3c-433c-a484-7f653c82ff1b)

Figure 3: Public Health England recommendations for removal (donning) of personal protection equipment (https://images.app.goo.gl/AJ2S2cXZrWqXqsJq6)

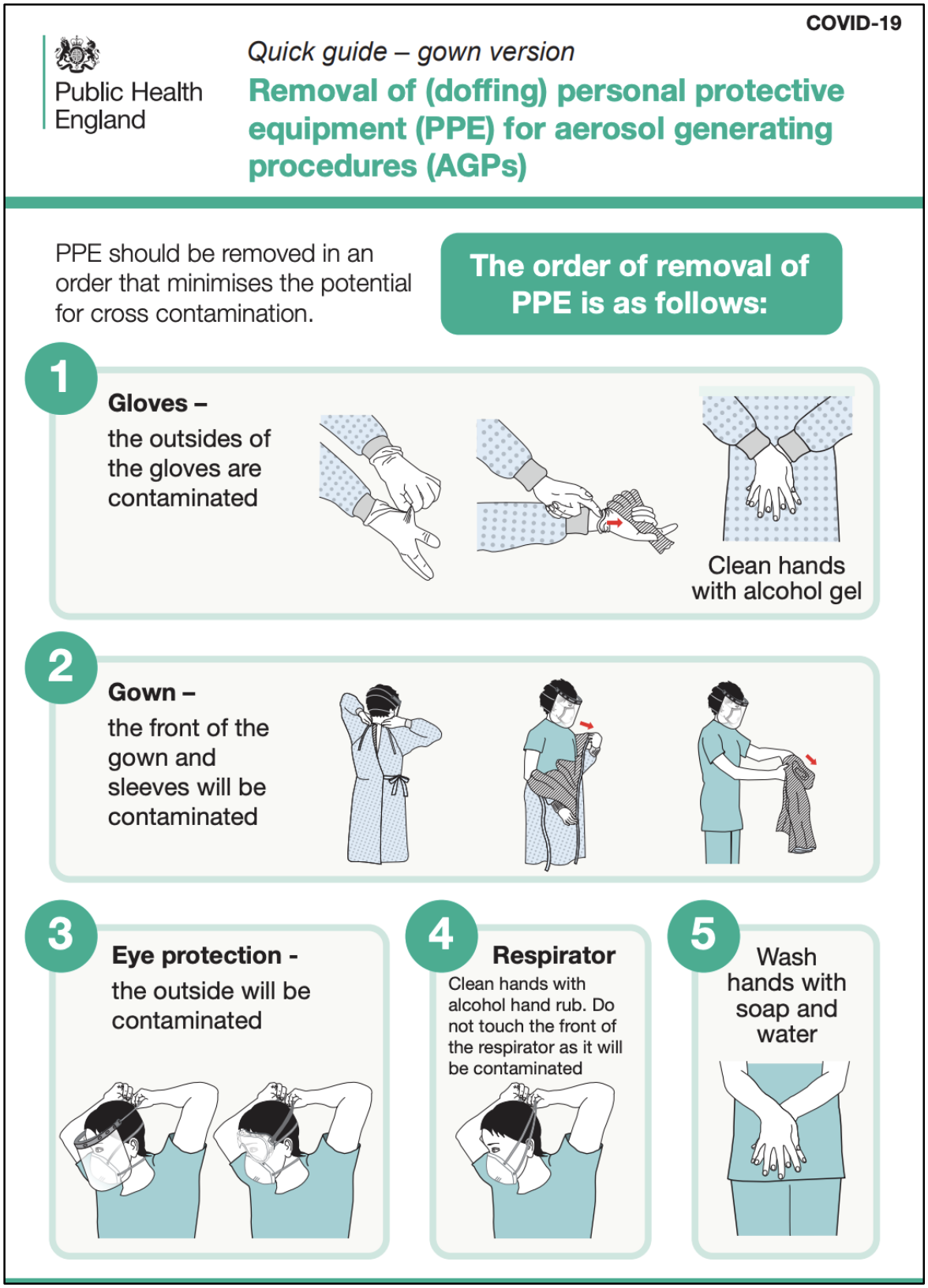

Figure 4: Public Health England recommendations for removal (doffing) of personal protection equipment (https://images.app.goo.gl/AJ2S2cXZrWqXqsJq6)

5. Clinical Significance

The review article highlights the importance of saliva as both a diagnostic tool and a major mode of transmission of the COVID-19 virus. Saliva isn't only a diagnostic tool for COVID-19 infection but also plays a key role in detecting systemic diseases, dental problems, genetic analysis, and forensic medicine. There have been innovative advances in

the development of salivary diagnostic tools, such as the identification of the salivary Proteome, Nano biochip technology, nano sensor test, OraQuick (antibody test), and DNA-based saliva tests, such as MyPerioID, OralDNA, and MyPerioPath.[33] The development of oral biosensors (Titanium, Urea, Alpha Amylase) has been a great innovation in this field.[34]

6. Conclusion

Saliva is a major factor in the transmission of the COVID-19 virus; at the same time, it's an important diagnostic tool to detect the virus. The development of sensitive diagnostic kits to detect the potential virus in the early stages of infection will be highly beneficial to dental practitioners and hospital clinicians. The recommendations suggested in this article are high-level guidance to the dental practitioners to combat the transmission of the COVID-19 virus effectively. The salivary diagnostics mark a new era in prevention and precision medicine. However, the cost of the development of new assays and devices would be on the higher end and would require acceptance by companies.

7. Source of Funding

None.

8. Conflict of Interest

None.

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