## Review article:

# COVID-19 Detection by Salivary Analysis: Easy and Reliable Approach in Massive Outbreak Dipayan Mojumder<sup>1</sup>, Satabdi Paul<sup>2</sup>, Ruman Banik<sup>3</sup>, Hasina Mahmuda Ferdushi<sup>4</sup>

#### **Abstract:**

Coronavirus disease (COVID-19) caused by 2019 Novel Coronavirus (2019-nCoV) recently emerged from Wuhan, Hubei province, China in December 2019. It showed devastating nature and already involved almost all the territories all over the world. It is a contagious disease and rapid, reliable diagnosis is needed to take measure for decreasing the infection rate. For detection of COVID-19 nasopharyngeal swab, sputum, broncho-alveolar lavage, saliva and other body fluid identified as useful samples. The aim of this review from recent studies is to explore the possibility of salivary analysis to identify COVID-19. Although nasopharyngeal swab is taken for rRT-PCR test as a worldwide accepted method, but it is a technique sensitive procedure needs expert personnel and adequate protection is necessary to avoid contamination. In the contrary, saliva sample can be taken easily by the patient himself at home which is a low-risk procedure and economical. So, in the pandemic of COVID-19, salivary analysis could be a good option for detection. Future study on this might explore the possible diagnostic capability of salivar for different diseases and underlying mechanism to find out the pathway for targeted drug therapies.

**<u>Keywords:</u>** COVID-19, SARS-CoV-2, saliva analysis, coronavirus infection, massive outbreak.

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#### **Introduction:**

Coronavirus disease (COVID-19) was first detected in December 2019 in Wuhan, Hubei province, China caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) or 2019 Novel Coronavirus (2019-nCoV).¹After that, it showed life-threatening nature and has been spreading throughout the world. According to World Health Organization (WHO), till now it has been affected 216 countries, places or territories and more than 4.5 millions of people all over the world.<sup>2</sup>The virus has shown its similarity with Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) and Middle East Respiratory Syndrome Coronavirus (MERS-CoV) and considered as third coronavirus of bat origin having human to human transmission and familial clustering.<sup>3,4</sup>

Clinical features of COVID-19 are fever, cough, shortness of breath, muscle ache, headache, confusion. Other reported features are sore throat, rhinorrhea, chest pain and even diarrhea. Some patients have fatal symptom such as Acute Respiratory Distress Syndrome (ARDS).<sup>5</sup>

COVID-19 is a contagious disease and those, who does not keep up social distancing and come in contact with patient without protective measures like N95 mask, face shield or goggles might be infected easily through coughing, sneezing, inhalation of droplet and contact through mucous membrane of nose, mouth, eyes and saliva.<sup>6</sup>

Saliva is a diagnostic body fluid and salivary biomarkers have the capacity to detect oral malignancy, periodontal problem, caries, diabetes and even lung cancer. Biological markers such as

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Hepatitis C virus RNA, Dengue virus specific IgA, human antibodies against Measles and Mumps can be detected in saliva.<sup>7</sup> In addition to that detection of HIV and Zika virus by simple saliva test is also reported.<sup>8, 9</sup>Certain respiratory virus including SARS-CoV has been identified in throat wash and saliva.<sup>10, 11</sup> Recently SARS-CoV-2 was isolated in saliva in a study indicating the possibility of salivary analysis could be an option to check the outbreak.<sup>12</sup>

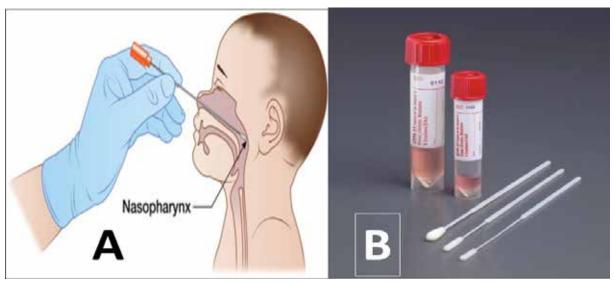
Rapidly spreading COVID-19 should be diagnosed quickly due to its deadly nature, for example in USA, more than 2000 people die everyday affected by this now. Saliva sample is easy to collect and procedure is economical comparative to the nasopharyngeal swab due to use of Personal Protective Equipment (PPE) is not necessary all time. The aim of this review is to explore the possibility of saliva sample analysis as a quick and reliable method for detecting COVID-19 disease.

#### **Discussion:**

According to WHO, for the detection of COVID-19 from suspected patients, nasopharyngeal and oropharyngeal swab should be taken in case of upper respiratory tract and in lower respiratory tract sample taken as sputum (if produced) and broncho-alveolar lavage or endotracheal aspirate when patients is in the severe respiratory disease. Sample must be taken by trained staff and collection should send immediately to the laboratory for Real Time Reverse Transcriptase Polymerase Chain Reaction (rRT-PCR) and confirmation given as

positive.<sup>13</sup>Althoughr RT-PCR test for detection of COVID-19 has highest sensitivity about 78.2% alone and combined with CT scan 91.9%, it is still worldwide accepted method for diagnosis of COVID-19.<sup>14</sup> However, taking sample from suspected patient may hazardous for both patient and healthcare workers. It is a matter of uneasiness and there is a risk of bleeding and injury to the soft palate or back of the nose if patient has any bleeding disorder such as thrombocytopenia.<sup>4</sup> Moreover, taking samples need close contact with the patient may create a vulnerable situation for staff (Figure 1).

Considering all these things, we can avoid such difficulties by salivary analysis. According to a recent clinical study in Wuhan, China 29% of the total hospitalized COVID-19 infected patients are healthcare staff. 15 So, it is a prime concern to prevent unwanted infection of doctors, nurses and others with SARS-CoV-2 as fighting with corona is unimaginable without them. Sometimes few patients cannot give nasopharyngeal specimen due to severe respiratory distress and even cannot produce cough.One case series reported only 28% of COVID-19 positive patient can produce sputum.16 For saliva sample, patient can cough out of saliva in a wide mouth container by himself at homeand sample can be taken easily to the laboratory for analysis and thus we can avoid contamination to surrounding environment. Additionally, this non-invasive procedure requires less isolated special room for receiving sample



**Figure 1:** Collection of nasopharyngeal specimens: (A) Swab is taken from above the soft palate at the back of the nose. It is a technique sensitive procedure and need trained personnel. (Picture taken fromwww.researchgate.net/profile/Kim\_Hare)(B) Dacron or Polyester flocked swab stick with container. (Picture taken from <a href="www.interpath.com.au/images/products//92/92-main-tn.ipg">www.interpath.com.au/images/products//92/92-main-tn.ipg</a>)



**Figure 2:** Saliva collection: (A) Easy and risk free sample taking procedure by patient himself even at home (Picture taken fromwww.who.int/csr/disease/plague/collecting-sputum-samples.pdf) (B) Wide-mouth sterile container for saliva. (Picture taken fromwww.deltalab.es/en/productos/sampling-and-transport/containers)

and more cost-effective(Figure 2).

In this pandemic stage of COVID-19 infection, worldwide Personal Protective Equipment (PPE) shortage is a rising problem, as it has a crucial role for doctors, nurses to prevent being infected at the time of service. Manufacturers will not be able to fulfil the increasing demand of N95 masks, protective gowns, face-shield day by day.<sup>17</sup>If we use saliva samples for detection of COVID-19, it will reduce use of PPE and also workforce during sample taking and subsequently decrease infection rates.

Saliva contains biological markers like DNA, RNA, proteins for detection of some viruses as well as diseases. Respiratory viruses for instance SARS-CoV and MERS-CoV shows their presence in saliva in more than 90% cases in rRT-PCR test. In a study SARS- CoV-2 in saliva specimen shows significant result(91.7%) and another research indicated saliva as a more sensitive and viable alternative to nasopharyngeal swab. PResearchers from Rutgers University suggested that, saliva is a robust source of viral RNA for COVID-19 detection and performs almost equal to swab-based study.

Coronavirus-19 existence in salivary flow can be occurred by some possible mechanisms. Virus can come from lower respiratory or upper respiratory tract and mixes with saliva, from gingival crevicular fluid or from other salivary glands via

ducts.<sup>20</sup>As a result the virus can be detected in saliva after many days of hospitalization. One of the research reported that SARS-CoV-2 infection found of a patient's sample after 11 days of infection.<sup>1</sup> It can show us the importance of saliva analysis in follow up of COVID-19 patients.

Coronaviruses are the enveloped, single-stranded RNA virus having glycoprotein spike on the envelope. This is the virulent part to stick with the host cell receptor. SARS-CoV attaches to the ACE2 receptor and MERS-CoV binds to the DPP4 (Dipeptidyl peptidase 4) mainly. SARS- CoV-2 also uses ACE2 receptors for viral entry into human host cells.<sup>21</sup> A study on rhesus macaques showed that in the early stage of progression of SARS-CoV, salivary gland cells are the primary target cells.<sup>22</sup>Over the spike protein of SARS-CoV-2 there are many activation sites and they are activated by Furin, an enzyme which can be found in many human cells. For the proper attachment of viral membrane with the host cell membrane the cleavage of the spike protein by cellular proteases (also called priming) is needed and for that TMPRSS2(atype2transmembraneserineprotease) is the additional requirement.<sup>23</sup>Interestingly, Furin and TMPRSS2 has been found in salivary gland cells and it reflects the possibility of primary site for the virus in human body.<sup>24, 25</sup>Asymptomatic COVID-19 infected patient might have the infection only confined in the salivary gland and

thus the infection may not involve the respiratory tract. Moreover, re-infection can be possible from salivary gland as a reservoir of virus. These should be taken under consideration for future research by taking saliva sample from ductal fluid only.

Analyzing saliva sample has also some limitations. Till now, nasopharyngeal swab is widely accepted for detecting respiratory viruses than saliva sample.14 Although the reported difference in sensitivity is very low between nasopharyngeal swab and saliva<sup>18</sup>, but for some reasons it causes some difficulties. In many territories such as Africa, South-East Asia region a huge percentage of citizens live below poverty line and to cover the load of infected people government advised to maintain social distancing and to stay at home rather than institutional isolation.26It is very difficult to keep them at home if they become false negative. As a result, unconsciously they meet with others and infect more people. However, false positive result may create a socially insulting position and miserable life by stopping family income of small businessmen, hawkers, daylabourers due to lock-down. These aspects may create some problems but as a fastest, easy and non-invasive procedure saliva sample analyzing may outweigh all drawbacks.

#### **Conclusion:**

COVID-19 is amajor concern of whole world. It has been affected all types of business, communication sectors, food supply and even educational programs. The world is now passing a crucial time to combat with this unwanted enemy. In this time of massive outbreak, we should find out an easiest, quick and reliable method of detection of COVID-19 and infact, saliva sample analysis can be the top priority. Further exploration is needed to investigate potential disease diagnostic capability of saliva and underlying mechanism to find out pathway for targeted drug therapies.

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