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Review Article

Saliva: A potential specimen for COVID-19 testing

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ABSTRACT

COVID-19 presence worldwide has brought substantial changes in our thinking process. The conventional biological specimen in disease due to viral aetiology ranges from blood to cerebrospinal fluid, Broncho-alveolar lavage. Through, established means of specimen have high sensitivity, other options like using Saliva specimen for testing in a developing country like India may be cost effective. The COVID-19 pandemic is still an evolving condition and best way to mitigate the infection is to increase in testing per day with isolation of the diseased individuals. Hence, Saliva could serve as a potential specimen for COVID-19 testing moreover available literature from other countries is offering promising results.

1. Introduction

Human race have faced several pandemics. With each pandemic, we have learnt to tackle the things socially, scientifically, economically, culturally i.e. impacting each and every aspect of human life worldwide. Though each aspect is vital for sustainability of the race and have contributed significantly to contain the infection nonetheless, the scientific researches pertaining to diagnosis and disease management have made humans sail through these pandemics.

2. Study

Recent pandemic caused by Corona Virus Disease (COVID-19) have claimed thousands of life worldwide. Most of the cases were and still diagnosed presently with real time reverse transcriptase polymerase chain reaction (RT-PCR). The diagnostic yield of the RT PCR depends upon the biological specimen used. Most commonly nasopharyngeal swab (NPS) is used. The sensitivity of NPS RT PCR is 63%.[1] The risk of transmission of the virus to the health care workers (HCW) via aerosol transmission during taking samples is very high. Many modifications were suggested to minimise this spread however, reports of health care workers getting infected with virus is seen frequently.

With increase in our understanding of this novel corona virus, various biological specimens were subjected for sensitivity and specificity and different methods of collection have been on trial however, the risk of transmission to HCW remained high. It is now one the biggest challenges to the scientific community to minimise this risk. Hence, the approach could be by minimising or no involvement of HCW in the process of sample collection. Patient self-collection of sample is one of the effective aspect studied in some of the available literature however, it would not replace the HCW as patients may not take sample from desired site even after proper instruction.[2] The chances of falsely negative result would arise in such scenario apart from other reasons of getting false negative result due to low viral load and virus is not picked up while in sample collection.

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Various immune-chromatography based rapid antibody detection kits were developed giving results in few minutes and have been widely used across the world and the sensitivity and specificity of the kit based antigen test remained 92.9% and 96.2% respectively in one of the study, however the small sample size in same study could not conclude this findings around the world.[3] Though it is cost effective method with better patient compliance, its false positive results with low sensitivity in early infection make these kits non reliable when compared with RT PCR.[4] In RT PCR perspective, the biological specimen may pick the dead virus from asymptomatic individual, giving false positive results in a clinically asymptomatic individuals. Hence, a midway of less of HCW involvement and more sensitive and specific biological specimen needed to address the issue.

In its early guidelines, Centre for Disease Control and prevention (CDC) have recommended a Nasopharyngeal, Oro-pharyngeal, nasal mid turbinate, an anterior nares or nasopharyngeal/nasal wash or aspirate or sputum (non-induced) swab RT PCR (Real time Polymerase Chain Reaction) after collection by health care professional for the diagnosis of COVID-19.CDC also have specified use of the viral transport medium, Amies medium or sterile saline for the use of transport of these swab sample.[5] Apart from this, ID NOW COVID-19, a point of care testing [POC] based on the isothermal nucleic acid amplification technology was also used under emergency use authorization where RdRp RNA fluorescently labelled segment amplification is identified making result available in 5 minutes.[6,7] The guidelines also mentioned about the use of personal protective equipment[PPE] by the sample collecting health care worker along with maintaining distance of 6 feet for the other health care persons who are not directly involved in the sample collection.[5] Initially, diagnostic test for COVID-19 was done at CDC later it was started at designated laboratories.

In the modification to previous recommendation, in march 2020, CDC advised to take nasopharyngeal sample(NP) and if OP sample is collected, it should be put in the same tube of where NP sample has been put.[8] Stool as a diagnostic specimen has been used for detection of various viral aetiology, so hypothesizing its use in COVID-19 was considered however, the Virus mainly get excreted in late phase of the disease hence chances of false negative results were high hence CDC in an update released in April, 2020 has not recommended for the use for the same.[9,10] In the revised guidance document published in the May 11^{th} 2020, CDC allows use of antigen and serological tests (i.e. IgG, IgM) in the clinical specimen with recommended validated study.[11] In an another modification, CDC cleared that the serology testing is validated for research and surveillance purposes only.[12]

In the interim guidelines released by CDC in May, 2020 stated that IgM and IgG may appear in the blood of diseased person simultaneously in contrary to conventional finding of IgM appearance follow by IgG. CDC also stated that mere appearance of antibodies does not protect humans for reinfection as the concentration of antibodies needed to confer immunity against reinfection is not known moreover, some individuals does not develop antibodies after infection and even if present provides a temporary immunity.[13] In the same guidelines, CDC has specified the antigenic targets in novel Corona virus 2 spike glycoprotein(S) and nucleocapsid phophoprotein(N). The same document also mentioned that the serological testing should not be used to make decisions for admitting the patient or advising to return to work. Since IgA is the secretory immunoglobulin giving a local immunity against pathogens, various commercial kits based on IgA were marketed however, CDC in the same guidelines have restricted to IgA testing as very less information is available on the dynamics of the said immunoglobulin.[13]

In India, Indian council of medical research (ICMR) a body under Government of India through National Institute of Virology (NIV), Pune has been updating guidelines for diagnosis of SARS CoV2 time to time. In its early guidelines before implementation of nationwide lockdown to contain the infection mentioned E gene assay follow by confirmation RdRp and N gene assay of the clinical specimen.[14] In a modification to previous Standard operating Protocol(SOP) ICMR specifically mentioned about the E gene assay as a screening test and as well mentioned to perform influenza testing on the clinical specimen. In this modified SOP, ICMR has also mentioned to send the sample to ICMR, if any samples comes positive and only when sample is positive with ICMR NIV with confirmatory assay of ORF 1b, RdRp gene, E gene, N gene assay then only can be declared positive.[15] In respect to collection of the biological specimen ICMR had laid certain guidelines for the safety of the health care workers including use of PPE, regular use of disinfectant as per recommendation along with biosafety measure and ensuring of maintenance of cold chains for sample with triple layer packing.[16]

Recent speculations regarding the usage of Saliva for the diagnosis of COVID-19 with equivocal sensitivity and specificity provides new perspective in the detection of this novel virus.[17] Though there are plausibility of studies, no HCW are needed for the sample collection process moreover, no special training is required for the patient to collect the specimen on its own. This method is non-invasive, convenient and can be utilized as a tool for mass testing, number of tests per day can be increased where the system is already overwhelmed with limited number of tests and even as a discharge criteria for COVID-19 patients as virus remain in saliva despite of negative NPS PCR, making it more reliable biological specimen to control the infection.[18] United states Food and

drug administration(FDA) in May 2020 in a press release recognises the salivary sample for the COVID-19 testing.[19]

Case reports of COVID-19 diagnosed on computed tomography (CT) chest findings when NPS PCR negative are also reported.[20] So, in clinically suspected individuals salivary PCR can be a utility if NPS RT PCR is negative. Though literature mentions high sensitivity and specificity in the Broncho-alveolar fluid RT-PCR[21] it is practically not possible for each suspected patient to undergo bronchoscopy to get the specimen.

In a study by Azzi L. et al., Saliva RT PCR was used in 25 individuals and found it as a reliable utility in the diagnosis of COVID-19.[22] Another study by Pasomsub E. et al. compared RT PCR results of NPS and saliva and concluded that it could serve as a potential alternative to the standard invasive collection of NPS.[23] In another study, Wyllie AL et al. also supported the findings of Pasomsub et al. and suggested use of saliva in the diagnosis of SARS-CoV 2.[24] In a meta analysis by Czumbel LM et al., taking in account nearly 26 studies and revealing 91% sensitivity for saliva PCR in the diagnosis of COVID-19.[25]

To conclude, saliva PCR can be used as a reliable tool for the diagnosis of COVID-19. The procedure for collection of sample is non invasive, self-collection is possible with no risk to the HCW with nearly equivalent sensitivity that of NPS RT PCR.

3. Source of Funding

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4. Conflict of Interest

None.

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