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COVID-19 DIAGNOSIS: CHOICE OF CLINICAL SAMPLE

Microbiology

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KEYWORDS

BACKGROUND:
By late December 2019 in Hubei province of Wuhan, China a group of patients presented with pneumonia like illness. The virus identified for this disease was a novel corona virus, third in the list causing zoonotic CoV infections and seventh in the list causing human corona virus (HCoV) infections. The International Committee on Taxonomy of Viruses (ICTV) named it as SARS-CoV-2, a novel betacoronavirus. SARS-CoV-2 belongs to family *Coronaviridae* and order *Nidovirales*. It is a single stranded, positive sense enveloped RNA virus. This virus primarily targets the human respiratory system causing pneumonia which has caused massive pandemic with high mortality. Disease caused by SARS-CoV-2 is named Corona virus disease-19 (COVID-19). The continued spread of COVID-19 poses a public health challenge and on 30th January 2020 it was declared as global health emergency by World Health Organisation (WHO). A global pandemic was declared by WHO on 11th March 2020.[1]

Phylogenetic analysis of SARS-CoV-2 put it under *Sarbecovirus* subgenus (β lineage of β-CoV genus). The genomic structure of SARS-CoV-2 is large and bears a peculiar crown of spike protein (S). The virus has four structural proteins and 16 non-structural proteins (nsp1-16). The structural protein includes the spike surface glycoprotein (S), small envelope protein (E), matrix protein (M), and nucleo-capsid protein (N). Non- structural protein includes the helicase and RNA-dependent RNA-polymerase (RdRp). The spike protein plays a pivotal role in viral entry and has two subunits (S1, S2) amongst which S1 harbors the receptor binding protein. [2]

The dissemination of SARS-CoV-2 mainly happens through contact and respiratory droplets when an infected person coughs or sneezes. Another potential route of transmission may be faeco-oral as there is evidence of presence of COVID-19 virus in the gastrointestinal tract. Persistence of SARS-CoV-2 on surfaces up to hours and days is also demonstrated. [3]The standard incubation period of COVID-19 is just about 5.2 days. The duration of COVID-19 stretches from 6 to 41 days with average of 14 days which is considered as the period from symptom onset till recovery/ death. COVID-19 infection is usually seen in adult patients with median age between 34-59 years. The disease severity increases in older population (>60 yrs) and more in those having an underlying condition co-morbid condition such as diabetes, cardiovascular diseases. The customary symptoms of COVID-19 are fever, cough, shortness of breath and muscle ache,[4] though the virus might have greater preference for infecting the lower respiratory tract.[5]

Rapid, early and reliable diagnosis is the key to contain the pandemic. Currently SARS- CoV2 nucleic acid detection is the gold standard for diagnosis of COVID-19. RT-PCR has few benefits as it is a specific qualitative assay with adequate sensitivity and it helps in diagnosing an early infection. Sensitivity and specificity of real time PCR depends on several factors including the choice of specimen. Proper sample collection, handling and storage of various samples along with nucleic acid extraction are key points to be taken in consideration to minimise errors.[6] ([T]he most commonly tested sample currently is nasopharyngeal swab which requires careful and meticulous sampling for a successful test.

In this comprehensive descriptive review we have discussed the possibility of using a variety of specimens such as nasal swabs, oro-pharyngeal swab, tracheal aspirates, sputum or lung tissue, blood and faeces etc. for diagnosis of a case of COVID -19. The sensitivity and specificity of each sample along with the ease of collection and transport etc. will be focus of discussion.

Nasopharyngeal (NP) swab:
SARS-CoV-2 clones in the upper respiratory tract and crest of infection is usually seen at the 5th day. The viral dispersing pattern resembles more to influenza virus in SARS-CoV-2 though the genomic similarity is close to that of SARS-CoV.[9] High viral load is found in the initial and escalating phase of the infection declining cautiously in the convalescence period [5, 10] Like other viruses causing upper and/ or lower respiratory tract infection, specimen collected from nose and throat are considered best for demonstration of virus/ its nucleic acid for diagnosis of Sars-CoV-2 infection too. In the current scenario nasopharyngeal swab is the most commonly used specimen and is considered the gold standard for detection of SARS- CoV-2.[7] NP swab should be collected using flocked swab for better yield. Collecting nasopharyngeal swab needs training of staff and may cause slight discomfort to patients. Swab should be inserted deeply in nasal cavity and twisted for 3 times and placed in viral transport media. The procedure presents a considerable risk to healthcare workers as the patient might cough or sneeze during sampling expelling virus particles.[8] Real time PCR for detection of SARS-CoV-2 RNA in NP swabs is considered as sensitive and specific method, however the sensitivity is relatively poor during early phase of infection.

Nasal swabs have higher sensitivity when tested 7 days before the onset of illness as compared to swabs taken 15 days after the onset of illness (50%) .[11] In contrast, studies which did serial testing of viral RNA in COVID-19 cases reported inconsistent viral presence during very early infection 2–6. [12-14] Most of the studies demonstrated low positivity of SARS-CoV-2 viral RNA in nasal and throat swab after 14 days of symptoms onset (27). [11]Zou et al also in their study found that SARS CoV2 viral RNA was faintly detected in nasal and throat swab after 14 days of symptoms onset.

The diagnostic accuracy of NP swabs is considered higher than that of OP swabs as NP swab has higher viral load which can be best demonstrated in paired sample taken on day 0-9 of symptom onset. (28)[15] SARS CoV-2 virus was detected in 63% of NP swabs in comparison of 32% of OP swabs of Covid-19 patients [16]

Oro-pharyngeal (OP) swabs:
Oropharyngeal swab is also a recommended sample for COVID-19 disease. Sample collection of OP swab requires the health care worker to properly inspect the patient's oropharynx and this is a risky procedure as it carries a high risk of transmission of infection. The sensitivity of oropharynx sample in early infection i. e. within a week of symptom onset is less (60% & 32%) as compared to samples taken from nasal cavity (73% & 63%).[17] A similar study also found the sensitivity of OP swabs declined after 15 days of symptom onset. (24))[16] Sample collection of OP swab alone is not recommended but if collected should be combined with NP swab and preferably within a week of illness for better diagnostic accuracy.[18]

Saliva:
Saliva is a promising candidate for SARS-CoV-2 diagnosis because the nature of collection is non invasion, more acceptable for patients as they can easily self-collect the sample and it is less hazardous for health care workers. It was found that detection of sensitivity from saliva sample of SARS-CoV- 2 is akin to nasopharyngeal swabs in early phase of infection.[19] Three methods are recommended to collect

saliva by coughing out/ deep throat saliva, swab for saliva and directly from salivary glands.[20] Amongst all three methods deep throat saliva has highest diagnosis rate of COVID-19 [21] SARS-CoV-2 RNA viral load peaks during the first week of symptom beginning (n=20/23), and declining over a period of time of 11 days. [20] Saliva would rather be a favored sample over nasopharyngeal or oropharyngeal samples when viral load is kept a track of, because it brings down the tenderness to the patient and health risks to healthcare workers during recurrent sample collection.[21]

Nasal swab:

Nasal swabs can also be used to collect sample for diagnosis of COVID-19 as it is a convenient alternative method of sample collection which can be self-collected or by a health care professional. The sensitivity and specificity of SARS-CoV-2 RNA detection from nasal secretion is 89.2% and 100% respectively, nearly equivalent to detection using nasopharyngeal swabs which is considered gold standard.[5] The viral load is higher in nasal swabs as compared to throat swab in early days of symptoms onset. [16] Nasal samples can be put to use to screen COVID-19 infected patients when nasopharyngeal swabs are not available.

Sputum:

Sputum samples can easily be used for detection of COVID-19 as it can be self-collected and does not require any special requirement of PPE. Proper education to the patient should be provided as to differentiate between sputum and oral secretions (saliva).

Concentration of viral RNA in sputum declines slowly, viral load peaks around 5-6 days after symptom onset and viral shedding persists till 28 days of symptom onset. *Roman Wölfell et* al in their study observed sputum samples collected between 4-9 days of symptom onset had active replication of virus with viral load as high as 2.35×10^9 copies/ml so the perceptible viral load turns on upon the day of symptom start.[22]

During the first 14 days of symptom onset SARS-CoV-2 virus could be noticed in sputum samples come after nasal swabs while throat swab was unpredictable after 8 days of onset of symptom.[23] The prolonged viral shedding in sputum is relevant not only for the control of infections in hospitals, but also for discharge management. [22] Also a study in Germany documented a high viral load of 108 copies per/ml in sputum sample of an asymptomatic patient five days after symptoms onset.[24] In critical situation where there is shortage of beds in hospital, early discharge with home isolation can be considered for patients beyond 10 days of symptoms and viral RNA load of > 100,000 copies/ml of sputum. [22]

Lower respiratory tract specimen:

Sample collection in lower respiratory tract, other than sputum may be bounded to patients attending with critical complaints and taken in the hospital. The collection of BAL fluid or endotracheal aspirate requires a well-trained health care professional with proper PPE as both are aerosol generating procedures with high risk of acquiring the disease. Ideally BAL or ET should be used for collecting lower respiratory tract specimens as the yield of SARS-CoV-2 viral RNA is quite high for diagnosis of COVID-19 disease. Few cases of SARS-CoV-2 early infection were missed by saliva/ NP/ OP specimens but their repeat sample collected from lower respiratory tract confirmed COVID-19 diagnosis. [25]

The detection of SARS-CoV-2 RNA in BAL fluid is quite high (44/15; 93%) followed by sputum (72/104: 72%). [26] In patients with progressive or severe conditions strong recommendation of collection of lower respiratory samples is required as ACE2 receptors are mainly distributed in alveolar type 2 epithelial cells and may contain high viral load. [19,27] These patients show high viral load along with prolonged viral shedding in lower respiratory samples as compared to upper respiratory specimens. Lower respiratory tract samples have high viral load and they may be required to assess the true viral clearance in patients. [28]

Faeces

Faeces can be a potential candidate for diagnosis or discharge policy of COVID-19 positive cases as there is evidence supporting the fact that patients with SARS-CoV-2 infection have gastrointestinal symptoms and presence of viral RNA in faeces. [29] Researchers found that patients positive for SARS-CoV-2 virus by NP swab also showed presence of virus in faeces sample. After repeated testing 8 patients were repeatedly positive for rectal swabs even after NP swabs were negative.[29] In another study presence of SARS-CoV-2 RNA in stool sample of 39 patients was detected and 17 amongst these 39 patients continued to shed viral RNA in stool even after becoming negative in respiratory samples. Viral shedding of SARS-CoV-2 viral RNA in faeces sample is till 27.9 days i.e 11.2 days longer than respiratory samples. (15)[10] Yang et al in their study reported that rectal swab positivity increases fifth day of disease (75%) as compared to oral swab. So, they concluded in their study that likelihood of viral dispersion in stool samples is for approximately 5 weeks more following respiratory samples become negative for SARS CoV-2 RNA. [10,30]

It is highly advocated to routinely test the stool sample with real-time RT-PCR, even after the clearing of viral RNA from the respiratory samples of the patients. So, the favoured method for SARS-CoV-2 detection in progressive COVID-19 infection cases feasibly be RT-PCR, of rectal swab to improve the sensitivity and reduce false negative results.[25]

Blood & serum:

Samples from blood can be used for nucleic acid testing and serological testing both. Few studies have detected viral RNA in blood (3/307, 1%) and 40% (6/15) of COVID-19 patients who despite of receiving medical treatment and negative RT-PCR of oral swabs tested positive with blood specimen.[9] In a research study, SARS-CoV-2 viral RNA was detected in 15-30 % of blood samples. Viral RNA of COVID-19 was found in both plasma (50%) and serum samples (78%) in first week of illness. [21] It could be effectual way to see the viraemia levels in the patient. High viral load is serum corresponds with lesser oxygen saturation along with mechanical ventilation and death. [21]

CONCLUSION:

The current crisis of the COVID-19 disease pandemic has emphasised on early diagnosis of infection to patients suffering from serious illness to curb the spread along with providing appropriate medical treatment. Currently a nasopharyngeal swab is endorsed for initial diagnosis or screening as it imparts higher diagnostic yield but it can miss early infection and is inconsistent for serial testing.

Current strategy for detection of viral RNA for SARS CoV 2 only in NP/OP swabs is not ideal. In a highly suspicious patient with negative NP/OP swab a repeat sample from multiple sites other than upper respiratory tract can be considered.

TABLE: Various samples used for diagnosis of Covid-19

Specimen	Ease of collection	Possibility of self-collection	Sensitivity	Ease of processing	Storage quality
Nasopharyngeal swab	Moderately Difficult	Not possible	71-98%	Easy	<5 days: 4°C >5 days: 70°C
Oropharyngeal swab	Moderately Difficult	Not possible	32%	Easy	<5 days: 4°C >5 days:70°C
Nasal swab	Easy	Possible	63%	Easy	<5 days: 4°C >5 days:70°C
Saliva	Easy	Possible	91%	Easy	<48 hours: 4°C >48 hours:70 °C
Sputum	Easy	Possible	74-88.9%	Easy	<48 hours: 4°C >48 hours:70 °C

Lower respiratory tract (BAL, ET)	Highly Difficult	Not possible	93-100%	Easy	<48 hours: 4°C >48 hours:70°C
Faeces	Moderatel y Difficult	Possible	29%	Difficult	<5 days: 4°C >5 days:70°C
Blood/serum	Difficult	Not possible	1-3%	Easy	<5 days: 4°C >5 days:70°C

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