

Confirmatory and supportive laboratory investigations in SARS-CoV-2 infection; a systematic review



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Received: 14 June 2020

Accepted: 29 June 2020

ePublished: 6 July 2020

Keywords: COVID-19, SARS-CoV-2, Coronavirus, Confirmatory tests, Supportive tests

Abstract

The pandemic of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread over 210 countries. In order to mitigate the epidemic and to flatten the curve, apart from multiple containment measures, an accurate identification of those infected is imperative. The objective of this review was to review, systematically, the performance of diagnostic and screening technologies and the yield of different clinical samples for the detection of SARS-CoV-2. Here we have critically evaluated the results obtained from different clinical samples and laboratory investigations and the performance of rRT-PCR (real-time reverse transcriptase polymerase chain reaction) technique in the diagnosis of SARS-CoV-2.

Citation: Silva EH, Lekamwasam S. Confirmatory and supportive laboratory investigations in SARS-CoV-2 infection; a systematic review. J Prev Epidemiol. 2020;5(1):e03. doi: 10.34172/jpe.2020.03.



Introduction

An outbreak of severe acute respiratory syndrome (SARS) due to coronavirus was reported from Wuhan, a town in Hubei province of China on December 12, 2019 (1). The epidemic was traced to Huanan seafood market in Wuhan and immediate genomic studies conducted using clinical samples from infected subjects revealed that the causative organism is of bat origin (1). The World Health Organization (WHO) declared the outbreak as a public health emergency first, and later as a pandemic. The common presenting symptoms of coronavirus disease 2019 (COVID-19) include fever, dry cough, shortness of breath, diarrhoea and vomiting (1) although uncommon presentations such as myocarditis and sudden death were also seen. At the time of writing this review (April 11, 2020) COVID-19 caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread over 210 countries, infecting nearly 1.7 million people and causing over 100 000 deaths, globally (2). In Sri Lanka, since the detection of first case on 11th February 2020, 197 positive cases have been reported with 7 deaths up to date. In order to prevent rapid spread of infection island-wide and to flatten the curve, the relevant authorities have launched an extensive search to trace contacts and isolate them.

The accurate diagnosis of COVID-19 is crucial since the symptomatology of the disease is not pathognomonic and can be seen in upper respiratory infections caused by many other pathogens. Furthermore, early diagnosis facilitates the isolation of COVID-19 patients from other patients to prevent cross infections and also health care workers to take adequate precautions in handling patients. Protecting frontline medical staff is a priority in a pandemic as the loss of workforce can weaken the health care services which are already overwhelmed by rapid patient turn over. Detecting COVID-19 antigen in respiratory tract secretions or aspirates is the most appropriate method to detect infection early. Detection of specific antibody (IgM) against COVID-19 requires minimum of 7 days from the time of infection limiting its use in the acute management of patients. Certain degree of uncertainty, however, exists among clinicians regarding the performance of antigen detection methods and the most appropriate samples used in the diagnosis of COVID-19.

The objectives of this review were to critically assess the evidence related to the performance of different diagnostic or screening tests and clinical samples in the detection of SARS-CoV-2

Core tip

This review will be helpful in making decisions on laboratory diagnosis of SARS-CoV-2 infection including sample collection. Further, the findings of this review can be used for future studies and medical education related to SARS-CoV-2 infection and any possible viral outbreak expected in future.

Methods

The systematic search was conducted in PubMed using the search terms, ["Coronavirus" or "Covid-19" or "SARS-Cov2"] and ["Diagnostic methods" or "Screening methods"] and a total of 407 articles were found. Separate searches were done in the OVID and EMBASE using similar methods. "Similar articles" facility in PubMed and the citations of previous reviews were used to check for missing articles.

The steps described in the PRISMA guidelines (3) were followed during this review. All the articles were screened by the two authors independently in blinded manner using a set of selection criteria. Uncertainties were resolved after discussions and 30 original articles were selected for the review (Figure 1, Table 1).

Inclusion criteria: Original research on laboratory investigations, English language publications, human studies

Exclusion criteria: Reviews, clinical trials.

PCR for COVID-19 antigen

Real-time reverse transcriptase polymerase reaction (rRT-

PCR) is the diagnostic or the screening test commonly used to detect the SARS-CoV-2 in clinical specimens, currently (4). Different studies have reported the use of clinical samples from different body sites from infected individuals. These included nasopharyngeal swabs (5-8) nasopharyngeal aspirates (5), oropharyngeal swabs (4), urine (4,6) stool (6), serum (4), plasma, sputum (6, 8-10) throat swabs (8,10,11), broncho-alveolar lavage fluid (BALF) (12) and anal swabs (13).

Samples taken from the respiratory tract have given positive results in the initial stages of the infection in most of the studies (4,5). Serum and plasma were not positive for up to day 7th (4) and even day 20th (5) of the illness in some studies. Serum from one of the two cases in Korea reported to be positive only on the day 8th, but the cycle threshold (ct) value was near the cut-off value (14). According to viral load kinetics, following the initial increase, viral load tends to decrease from day 7th in upper and lower respiratory tract (14). Further, in urine and stool samples, viral load has not been sufficient to be considered positive, despite the presence of mild diarrhoea in the patients (14).

Lo et al, have discussed the importance of using nasopharyngeal specimens for SARS-CoV-2 detection. In their study 90% of the cases were diagnosed using nasopharyngeal specimens. However, in one patient, diagnosis was confirmed using a sputum sample after a negative and an inconclusive result from NPS samples. In contrast to other analyses, authors reported 90% viral

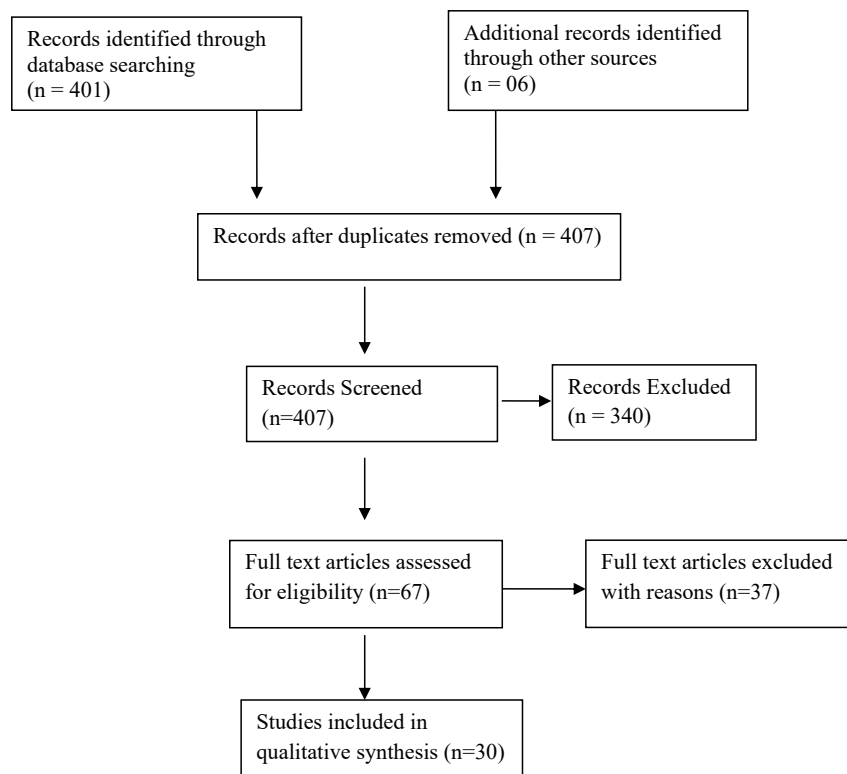


Figure 1. PRISMA flow chart showing the selection of articles for review.

Table 1. Summary of the clinical studies included in the review

Reference	Level of evidence	No. of patients	Symptoms presented	Samples tested	Test/s done
Wu et al ¹	Case report	1	Severe respiratory syndrome with fever, dizziness and a cough	BALF	Metagenomic RNA sequencing
Holshue et al ⁴	Case report	1	Fever, cough	NPS, OPS, Stool, Serum, Urine	rRT-PCR, CBC, Glu, BUN, Cr, TP, Al, TB, ALT, AST, ALP, Fib, LDH, PT, INR, CK,VL
Haveri et al ⁵	Case study	1	Runny nose, nausea, fever, weakness, cough	NPS, NPA, serum	rRT-PCR, Antibody response
Lo et al ⁶	Cross-sectional	10	Fever, diarrhoea, cough, dyspnea, sore throat, nausea, myalgia, rhinorrhea, nasal congestion, dizziness, abdominal pain	NPS, sputum, urine, faeces	qRT-PCR
Han et al ⁷	Case study	1	Fever, cough, white phlegm, stuffy and runny noses, vertigo, fatigue, chest tightness, nausea, bosom frowsty	NPS	rRT-PCR, CBC, CRP, Pr, AST, Tr, LDH, CK, Fib, Dd, Glu.
Chan et al ⁹	Family cluster	6	Fever, respiratory symptoms	NPS, TS, Stool, urine	rRT-PCR, whole genome sequencing, CBC,PT, INR, APTT, Dd, Fib, CRP, Al, TB, ALT, ALP, AST, Urea, Cr, Electrolytes, CK, LDH, Amy
Zhou et al ¹¹	Retrospective cohort	191	Fever, cough, dyspnoea, fatigue	TS	Next generation sequencing or rRT-PCR, CBC, PT, Cr, Al, ALT, LDH, CK, Hs-CTI, Dd, SF,IL-6, Pr
Liu et al ¹²	Cross-sectional	12	Fever, cough, myalgia, diarrhoea	TS, BALF	rRT-PCR, CBC, ALB, CRP, LDH, CD8, CK, Myo, CTI, BNP, CK-MB
Chen et al ¹³	Retrospective	57	Fever	PS, blood, AS	rRT-PCR
Kim et al ¹⁴	Cross-sectional	2	Fever, chills, myalgia, sore throat	NPS, OPS, urine, stool, serum, plasma	rRT-PCR
Zou et al ¹⁶	Cross-sectional	18	NR	NS, TS	rRT-PCR
Li et al ¹⁷	Case report	2	Fever, dry throat, difficulty breathing, nausea, vomiting, diarrhoea	TS	rRT-PCR, CBC
Zhang et al ¹⁹	Retrospective	95	NR	NS, PS	CBC, CRP, Dd, ALT, AST, Cr, α -HBD, LDH, CK, TP
Chen et al ²⁰	Retrospective	99	Fever,cough, shortness of breath, muscle ache, confusion, headache, sore throat, rhinorrhoea, chest pain, diarrhoea, and nausea and vomiting	TS, sputum, endotracheal aspirates	rRT-PCR, CBC, APTT, PT, Dd, Al, ALT, AST, TB, BUN, Cr, CK, LDH, Myo, Glu, Pr, IL-6, ESR, SF, CRP
Chen et al ²⁴	Case study	2	Fever, cough	BALF	Metagenomic next-generation sequencing, CBC, Al, AST, CK, CK-MB, LDH, Urea, Cr
Lan et al ²⁸	Cross-sectional	4	NR	TS	rRT-PCR
Zhou et al ²⁹	Cross-sectional	7	Severe pneumonia	Oral swabs, AS, BALF, serum	Metagenomic RNA sequencing

BALF; broncho alveolar lavage fluid, NPS; nasopharyngeal swab, OPS; oropharyngeal swab, NPS; nasopharyngeal aspirate, TS; throat swab, PS; pharyngeal swab, AS; anal swab, NS; nasal swab, rRT-PCR; real time reverse transcriptase polymerase chain reaction, CBC; complete blood count, Glu; glucose, BUN; blood urea nitrogen, Cr; serum creatinine, TP; total protein, Al; albumin, TB; total bilirubin, ALT; alanine transaminase, AST; aspartate transaminase, ALP; alkaline phosphatase, Fib; fibrinogen, LDH; lactate dehydrogenase, PT; prothrombin time, INR; international normalized ratio, CK; creatine kinase, VL; venous lactate, qRT-PCR- quantitative reverse transcriptase polymerase chain reaction, CRP- C-reactive protein, CTI; cardiac troponin I, Pr; prolactin, Tr; troponin, Dd; D-dimer level, Amy; amylase, Hs-CTI; high sensitivity cardiac troponin I, SF; serum ferritin, IL-6, interleukin 6, Myo; myoglobin, BNP; brain natriuretic peptide, α -HBD- alpha hydroxybutyrate dehydrogenase, ESR; erythrocyte sedimentation rate.

positivity in faecal samples of these patients. Moreover, in one patient, the virus has been detected in faeces up to 14 days from the onset of symptoms (6). This observation was in par with the first case reported from the United States (4) but in contrast to the observations made by Kim et al (14). Apart from the respiratory tract, SARS-CoV-2 has an increased affinity for gut mucosa due to the expression of cell receptor angiotensin converting enzyme II in absorptive enterocytes in the ileum and colon (15). Some have suggested testing NPS and faecal samples simultaneously to improve the detection rate so that infected subjects are not released to the society (6). In addition, it is also important to know whether the anal tract is a possible transmission route for the virus as viral

shedding was common in faeces although viability of the virus is unknown (6). Further it is evident that in some cases virus RNA conversion in the stool sample is delayed (6). Therefore, patients with negative NPS and positive stool samples may be undergoing the convalescent phase but they may remain infective (6).

Liu et al, comparing the results of throat swabs and BALF in three patients, found BALF to give positive results while throat swabs were negative, indicating that BALF may be more reliable than throat swab for the detection of SARS-CoV-2 (12). However, they also report a patient in whom BALF was negative while throat swab was positive (12).

After analysing viral load in 17 throat and nasal swabs of symptomatic patients, Zou et al found nasal swabs to

have higher viral load compared to throat swabs. These samples have been obtained soon after the onset of symptoms and the authors have suggested that viral RNA shedding of SARS-CoV-2 resembles that of influenza but different from SARS-CoV-2 (16). The false negative results observed by Dasheng et al, may be due to the low viral load present in the throat swabs (17). These findings highlight the importance of using the most suitable specimens for viral detection and also the need of analysing samples from multiple sites.

Chen et al have studied viral replication in extrapulmonary sites of the body and whether patients with severe symptoms show difference in virus replication and distribution compared to patients who exhibit milder symptoms (13). Blood, pharyngeal swabs and anal swabs were collected from confirmed COVID-19 cases and patients were divided according to the symptoms as mild and severe (13). In this analysis, compared to blood negative group, blood positive group showed severe disease. The viral RNA positivity in anal swabs also showed a significant positive correlation with disease severity. Once again these findings indicate that the digestive tract is an extrapulmonary site where viral replication takes place. Authors have proposed that increased viral load in the lungs can be released to blood via damaged alveoli, resulting viral spreading throughout the body and later re-infecting the digestive tract through ACE-2 (13). The detection of viral RNA in extrapulmonary sites is a serious concern as these patients would require extra precautions in nursing (13). However, some researchers have claimed that the presence of viral RNA in extrapulmonary sites does not necessarily indicate the presence of the infectious virus in those sites (4).

Differential diagnoses

Studies from Italy (18), United States (4), and China (9) have highlighted the importance of considering alternative diagnoses in patients suspected of SARS-CoV-2. Bordi et al, have investigated 126 suspected cases in Italy, using a rapid molecular test (QIAstat-Dx respiratory panel (QIAGEN, Milan, Italy)) and found that 53.2% of patients were positive for respiratory pathogens (viral or bacterial) other than SARS-CoV-2. Influenza virus was the commonest among suspected individuals and only three out of 126 were tested positive for SARS-CoV-2 by rRT-PCR technique (18). Screening suspected cases with broad spectrum diagnostic panel is of immense help in clinical management and making containment decisions on patients especially during an outbreak (18).

Other laboratory investigations

Other laboratory abnormalities seen in patients with SARS-CoV-2 include leukopenia (4), leucocytosis (11), lymphopenia (6,7,11,12) mild thrombocytopenia (4,9,19), hypoalbuminaemia (12), hypoproteinaemia (19), decreased CD 8 count (12), elevated creatinine kinase (CK) (4,11,19),

alkaline phosphatase (ALP) (4), alanine aminotransferase (ALT) (4,19), aspartate aminotransferase (AST) (4,19), lactate dehydrogenase (LDH) (4, 6, 9, 12,19) d-dimer (DD) (11,19), serum ferritin(SF) (11), high sensitivity cardiac troponin I (11) (hs-CTI), Interleukin-6(IL-6) (11), prothrombin time(PT) (11), procalcitonin (Pr) (11), creatinine(Cr) (11,19), C-reactive protein (CRP) (7,9,12,19,20), fibrinogen (9), activated thromboplastin time (APTT) (9), α -hydroxybutyrate dehydrogenase (α -HBD) (19).

Zhou et al revealed that leucocytosis, lymphocytopenia, elevated ALT, LDH, hs-CTI, CK, DD, SF, IL-6,PT, Pr and Cr were associated with increased mortality. In addition, DD levels higher than 1 μ g/mL on admission were also associated with increased likelihood of death, indicating that disruption of coagulation process is a surrogate of severe disease (11). In addition, baseline lymphocyte count of survivors has been significantly higher compared to the non-survivors. Further, the lowest lymphocyte count was seen on the day 7 among survivors and improved afterwards whereas in non-survivors lymphopenia persisted until death. Moreover, in the non-survivors, a rapid increase of hs-CTI was observed from day 16 (11).

Li et al reported a patient who had normal leukocyte counts and increased lymphocyte count in contrast to the majority of the patients (17).

Correlations between viral load and serum albumin, and blood lymphocytic and neutrophil counts have been reported by Liu et al recently. Further they found lung injury to be associated with alteration of serum albumin, lymphocyte count and percentage, neutrophil percentage, LDH, and CRP (12). In addition, they found hypoalbuminaemia, lymphopenia, and elevated levels of CRP and LDH to be strong predictors of severe acute lung injury on admission (12).

When compared with patients with mild or moderate disease, patients with severe infection had higher AST, LDH and leucocyte and neutrophil counts. Further, serum CRP was associated with severe infection (6).

According to Zhang et al, compared to leukopenia, leucocytosis is more related to the severity of the disease and this is believed to be due to the excessive inflammation and tissue injury seen in severely ill patients. Further, neutrophilia was associated with pneumonia and composite clinical endpoints of SARS-CoV-2 patients. Even in the presence of leucocytosis, lymphopenia was a regular feature in these patients and this was believed to be due to the translocation of lymphocytes from peripheral blood to the lungs (19). Though viral RNA was detected in urine, significant difference was not found in serum creatinine between severe and non-severe patients. Elevated LDH, α -HBD and CK were found in 77.9%, 92.6% and 29.5% of patients possibly due to acute myocardial injury (19). Zhang et al, however, excluded patients with chronic underlying diseases in this analysis which could have an impact on the results of laboratory examinations (19).

Role of the genetic tests

In an outbreak of this nature, it is important to know the duration and the level of virus replication in order to assess the risk of transmission and to decide on the isolation procedures (11). Genomic sequencing or nucleic acid tests are considered the gold standard for detection of the viral infection (17). As viral RNA detection is more sensitive than virus isolation, almost all the studies have used qualitative or quantitative viral RNA detection tests as the marker of choice to diagnose SARS-CoV-2 infection (11). The commonly used tests for viral detection include rRT-PCR (5, 17) and qRT-PCR (12) tests. The target genes of the real time RT-PCR tests include the Envelope (E) (5, 21), the RNA dependent RNA Polymerase (RdRP) (5, 9, 21), nucleocapsid (N) (5, 12, 22), Spike (S) (9), and ORF1b (12,22) genes.

Corman et al have recommended using E gene assay for routine screening and RdRP as the confirmatory assay, as they have shown higher sensitivity. They have obtained less sensitivity for N gene (8).

Konrad et al have also concluded that E gene assay was more sensitive compared to the two RdRP assay combined with a system of one step RT-PCR. However, E gene assay has given more background signals disturbing the assay results (21).

Chu et al reported that the sensitivity of N gene assay was 10 times higher than the ORF1b gene assay (22). They have suggested that the increased expression of subgenomic mRNA in the clinical samples gives rise to higher N gene copies. Considering these results, it was recommended using N gene assay as the screening assay and the ORF1b assay for the confirmation (22).

In the study by Chan et al, all respiratory samples gave positive results with both RdRP and S genes with conventional RT-PCR and only for the S gene by rRT-PCR. The phylogenetic analysis of the PCR products revealed that the amplicon sequences of both the genes from all the positive patients were different from previously known coronaviruses of human or animal origin (9). However, complete genome sequences of two strains from two patients were identical to each other and resemble the bat SARS-related coronavirus reported in 2018 (9).

Interestingly, Pfeifferle et al have introduced a fully automated system called cobas 6800, an open channel, rapid system, to cope with the increased demand for testing. The advantages of this method include minimal staff training, rapid turnaround time and high sensitivity and specificity of the assay. Cobas 6800 seems to be an alternative for an outbreak where testing becomes a challenge (23).

Chen et al suggest the use of metagenomic next generation sequencing (MNGS), which allows the direct examination of the infectious organism from the clinical sample. The RNA based technique is capable of revealing the entire 'infectome' (RNA viruses, DNA viruses, bacteria, eukaryotes), rapid and accurate diagnosis of a pathogen

(24). In addition, Ai et al suggested a combined approach for SARS-CoV-2 detection using MNGS, rRT-PCR and clustered regularly interspaced short palindromic repeats (CRISPR) based assay (25).

False negative results of rRT-PCR

rRT-PCR has given false negative results in some instances. Dasheng et al reported false negative rRT-PCR test results in throat swab samples taken from two patients with clinical symptoms (17). In one patient two consecutive rRT-PCR tests were negative, and only the third sample has become positive (17). Insufficient specimens and erroneous laboratory procedures are the most likely reasons for these false negative (26). In addition, infection route, stage of the disease progression, specimen collection timing and method and coinfections may also contribute to false positive rRT-PCR results (17). Authors have highlighted the importance of considering clinical manifestations, laboratory investigations and radiological features in the chest CT in the diagnosis of SARS-CoV-2 infection to overcome the approximately 20% of false negative rRT-PCR results (17). The National Health Commission of China has revised the criteria for the diagnosis of SARS-CoV-2, with the introduction of 'clinical diagnosis', where diagnosis is based on pneumonia features detected on chest CT, irrespective of rRT-PCR test results (27). Further, rRT-PCR can be applied for making decisions on isolation and timing of discharge (17).

Positive RT-PCR results, however, have been seen in respiratory specimens obtained from recovered patients. This can be due to false negative test results, re-activation or viral residues in the respiratory tract. It is still unclear whether these patients are contagious it is important to adhere to containment measures (28).

Serological investigations

The serological diagnosis of the SARS-CoV-2 has been analysed in three studies. Zhou et al have used a previously developed nucleocapsid (N) protein from bat SARS-CoV Rp3 as antigen for IgG and IgM enzyme-linked immunosorbent assays (ELISAs). The viral antigen levels have shown a clear trend in IgM and IgG levels, in the days 7, 8, 9 and 18 from the onset of the symptoms in a selected patient. However, the IgM titre of the last sample has decreased. Further, after 20 days of disease onset, samples were positive for IgG. In addition, three IgM positive samples were also detected, indicating an acute infection (29).

In the second study, the viral antibodies were undetectable till day 4 from the onset of the disease. From day 9 to day 20, IgM antibodies rose from 80 to 320 and IgG from 80 to 1280 (5).

However, Xu et al reported that in a family cluster of six people, where five of six people were SARS-CoV-2 specific IgM positive but according to the molecular assay only two were positive. They have highlighted the importance

of performing serology tests for detection of SARS-CoV-2 (30).

Discussion

There is a paucity of large datasets related to laboratory testing in SARS-CoV-2 infection. This is understandable since it is a new virus detected nearly five months ago. Further, the most of publications are from China and small sample size is a limitation in most of the studies.

Due to small numbers, data is insufficient for a proper head to head comparison of the performance of different analytical methods. The same limitation is observed in deciding the best clinical sample for establishing the diagnosis of COVID-19.

Data related to COVID-19 pandemic is still building up and clinicians and health care providers need to base their decisions on the limited information available. This is clearly evident in therapeutic decision making. Although numerous antiviral medications have been proposed the scientific evidence is not robust to recommend a uniform antiviral treatment. Similar situation prevails in the investigation of patients, this review, however, will help clinicians involved in the care of COVID-19 patients to understand the uses and limitation of the current laboratory investigations of SARS-CoV-2.

According to current evidence, rRT-PCR appears to be the most appropriate method available for the detection of SARS-CoV-2 antigen. The technology is relatively inexpensive. Widely available and can be installed in temporary buildings particularly to facilitate community screening. NP samples have given high positivity particularly in symptomatic patients in the early stage in the illness. The procedure is simple, requires less training and can be done in the community. Bronchoscopic aspiration from the lower respiratory tract, although have given high positivity, can be reserved for selected patients who are negative in initial investigations but have a high clinical suspicion. Detection of viral antigen in stool samples is desirable especially when the presentation is delayed and NG samples have given negative results. Examining samples from multiple sites would increase the detection rate but this may have limitations due to cost considerations. When initial investigations are negative in a patient with suggestive symptomatology, the current evidence is not sufficient to make recommendations whether to repeat the testing of samples obtained from the same site or proceed to testing samples from multiple sites.

Conclusion

Samples from upper and lower respiratory tract shows increased positivity with SARS-CoV-2 compared to samples from extrapulmonary sites. However, throat swabs were not sensitive enough to detect SARS-CoV-2. Despite drawbacks rRT-PCR appears to be the most logical and practical assay that can be used for community screening. However, moving to fully automated systems or combined

approached may enhance SARS-CoV-2 detection.

Authors' contribution

EHS and SL contributed to data gathering. EHS prepared the primary draft and the final edit was conducted by SL. Both authors approved the final version for publication.

Conflicts of interest

All authors have no conflict of interest to declare.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the author.

Funding/Support

None.

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