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REVIEW

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Detection of SARS-CoV-2 using real-time polymerase chain reaction in different clinical specimens: A critical review

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Abstract
Coronavirus disease 2019 (COVID-19) is a disease caused by a new strain of coronavirus named as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). Globally, since the outbreak, more than seven million confirmed cases of COVID-19 have been reported. The rapid spread and increase in the number of new cases is due to person-to-person transmission. To further control its transmission, early laboratory diagnosis of both asymptomatic and symptomatic patients is crucial. Presently, the COVID-19 diagnosis of infected individuals is dependent on computed tomography scanning and real-time polymerase chain reaction (PCR). The latter is considered more sensitive and efficient for early diagnosis. In this review, general comparisons are made (cases, fatality rate, incubation period, clinical features, and reservoirs) and diagnostic laboratory procedures (specimens, extraction methods, and positive rates by real-time PCR) are compared between SARS, Middle East Respiratory Syndrome, and SARS-2. In total, 8982 SARS-2 suspected patients specimen data were retrieved, in which 40.9% (n = 3678) were detected as positive by real-time PCR. The specimen-wise high detection rate was observed from bronchoalveolar lavage, followed by saliva, nasal swabs, and sputum. As the COVID-19 cases are persistently increasing, the selection of appropriate specimens and laboratory assay would help in rapid and timely diagnosis.

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Introduction

Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is a beta-coronavirus.[1] This emerging COVID-19 outbreak is considered to be a constant threat for human health worldwide. Development of a vaccine for coronavirus-2 is crucial for controlling its rapid spread. However, the virus is evolving continuously due to climate change and increasing interactions between humans and animals.[2] The SARS-CoV-2 genome is composed of approximately 30 kb, single-stranded positive-sense RNA with a 5′-cap structure and 3′-poly-A tail.[1] Genomic sequencing and phylogenetic analysis have shown that SARS-CoV-2 is closely related to SARS-CoV-1 and Middle East Respiratory Syndrome coronavirus (MERS-CoV).[3] Most infections caused by human coronaviruses are clinically asymptomatic or show mild clinical symptoms with the exceptions of SARS-CoV and MERS-CoV.[4-6] SARS-CoV-1 is a beta-coronavirus that emerged in 2002 in China with more than 8000 confirmed human cases and 774 deaths in more than 37 countries from 2002 to 2003.[6] The main reservoir of SARS-CoV-1 was the civet cat.[7] MERS-CoV was first detected in Saudi Arabia in 2012 with a total of 2494 confirmed cases and 38 deaths.[8] Many cases of MERS-CoV were also recorded in South Korea.[9] The main reservoir of MERS-CoV was bats, with dromedary camels as the intermediate host. There were 904 confirmed MERS-CoV cases with 347 deaths in 2014. MERS-CoV infection was reported in 23 countries, including the Middle East, Europe, Southeast Asia, and North America (Table 1 and Table 2).[10]

Recently, on December 31, 2019, pneumonia-like cases were reported in Wuhan, Hubei province in China with unknown etiology.[1] Later on, this was identified as a Novel coronavirus that closely resembled SARS-CoV.[11] The virus spread throughout the world and on January 30, 2020, the World Health Organization (WHO) declared the outbreak as an international public health emergency.[12] Almost 7.0 million cases with 0.3 million deaths have been reported worldwide up to June 9, 2020. The highest number of cases are reported in the Americas (3,311,387 cases with 181,804 deaths), followed by European countries (Spain, Italy, Germany, and France) having 2,286,560 cases with 184,120 deaths, Eastern Mediterranean 641,429 cases with 14,602 deaths, South-East Asia (364,196 cases with 9970 deaths), Western Pacific (191,275 cases with 7112 deaths), and Africa (135,412 cases with 3236 deaths).[13]

Source and transmission of COVID-19

The source of COVID-19 is thought to be animals, and initial cases have been associated with the seafood market located in Hubei, Wuhan; however, the original cause has not yet been confirmed. Bats are considered the reservoirs of COVID-19,[14] as its genome is similar to that of bat coronavirus, with 85–96% homology.[15] The WHO and Center for Disease Control and Prevention (CDC, USA) have confirmed human-to-human transmission of the disease.[16-18] The transmission rate of SARS-CoV-2 is high due to the recombination of S protein in the receptor-binding protein (RBD) region, which enhances its transmission capacity.[19]

Clinical manifestations and diagnosis

The symptoms of COVID-19 include fever, myalgia, cough, dyspnea, fatigue, decreased leukocyte count, and radiological evidence of ground-glass lung opacities compatible with atypical pneumonia.[20] Infected patients also have various clinical courses in their respiratory, digestive, and genital organs, ranging from asymptomatic to severe symptoms.[21] In severe cases, organ dysfunctions, such as acute respiratory distress syndrome, acute cardiac injury, acute kidney injury, and even death, may occur.[22]

Patients with COVID-19 can be diagnosed only via radiological techniques, including portal chest X-rays and computed tomography scanning (CT-scan), and laboratory-based techniques. For laboratory-based detection, real-time reverse-transcription polymerase chain reaction assay (Real-Time RT-PCR)[23] can be used for confirmation. Some researchers and clinicians prefer CT imaging over RT-PCR to identify SARS-CoV-2 and determine its severity, as progressive peripheral ground-glass opacities in the lungs can still be observed even if the RT-PCR result is negative.[24] However, RT-PCR is the most efficient and sensitive technique and is considered a confirmatory and gold standard diagnostic test for viral respiratory infections.[1,3,12,25]

In this review, we have analyzed previous studies regarding coronavirus detection by RT-PCR. The objective of this review was to identify the role and sensitivity of RT-PCR in the detection of SARS-CoV-2.

Current literature

The lessons learnt from previous outbreaks of SARS and MERS have helped the scientific community to easily identify and detect COVID-19 within a few weeks of its emergence, whereas SARS outbreak in 2002 took approximately five months to be detected.[26] Whole genome sequencing was carried out to identify SARS-CoV-2, and sequenced data were utilized to design specific and sensitive PCR probes and primers. RT-PCR is currently regarded as the gold standard diagnostic method for COVID-19. However, the sensitivity of this method in throat swabs is approximately 59%.[27] RT-PCR has given negative results for patients

Table 1 Comparison of cases and mortality rate of COVID-19, MERS, and SARS.

Year	Disease	Region of origin	No. of countries	Total cases	Estimated deaths	References
2002	SARS	China	37	8000	774	6
2012	MERS	Saudi Arabia	25	3398	385	8,9
2019	COVID-19	China	205	6,931,000	400,857	13

Table 2 Comparison of reservoirs, incubation period, common clinical features, laboratory specimens, and diagnostic procedures of COVID-19, MERS, and SARS.

Name of disease	Primary reservoir	Intermediate reservoir	Incubation period	Lab specimen	Diagnosis	Common clinical features	References
SARS	Bats	Civet cat	4-5 days	Pharyngeal swab, blood, urine, feces, sputum, nasal samples	Radiography, PCR, autopsy	Fever, cough, breathing difficulty	6
MERS	Bats	Camel	2-14 days	Pharyngeal swab, blood, urine, feces, sputum, nasal samples	Radiography, PCR, autopsy	Fever, cough, breathing difficulty	8,9,20
COVID-19	Bats	Pangolin	2-14 days	Pharyngeal swab, blood, urine, feces, sputum, nasal samples	Radiography, PCR, autopsy	Fever, myalgia, cough, dyspnea, fatigue	21-23

suspected of being positive based on clinical symptoms and exposure history who had abnormal chest CT scans. In some cases, the RT-PCR result was positive even after the patient had fully recovered.[28,29] Studies have shown that RT-PCR can give false negative reports in patients with COVID-19.[30] This may be due to a low viral load in the pharyngeal swabs of some patients, inappropriate transport and storage of samples, and the relatively low sensitivity of RT-PCR.[31] The frequency and duration of shedding of SARS-CoV-2 in stools and urine are unknown.[32,33]

After collection, samples to be tested should reach a laboratory as soon as possible. During shipment, the specimens should be maintained at 2–8°C, and if sample delivery is delayed, viral transport medium should be applied. In case of further delay, specimens can be frozen to −20°C or ideally −70°C, and repeated freezing and thawing should be avoided.[34]

RT-PCR has been used widely for the identification and quantification of various pathogenic viruses, such as MERS, SARS, and Zika virus.[35] However, RT-PCR for the diagnosis of COVID-19 is time-consuming and involves a laborious sample processing step, thereby limiting its use.[32] In routine confirmation of COVID-19, detection of a unique RNA sequence is carried out by RT-PCR (Table 3). Viral genes targeted so far include *N*, *E*, *S*, and *RdRP* genes.[33]

The sequence regions ORF1b/RdRp and N (*N1*, *N2*, and *N3*) are highly conserved among the sarbecoviruses and are thus used to design sequence-specific primers and probes. The expected amplicon sizes of *ORF1b* and *N* gene assays are 132 bp and 110 bp, respectively.[15] The "SARS-CoV-2_N2, N3" of the USA and the "ORF1ab" of China are the most sensitive primer-probe sets for *N* and *Orf1* genes, respectively.[17] The China National Institute for Viral Disease Control and Prevention, Beijing, has designed primers and probes for *N* and *RdRp* genes.[36] The School of Public Health, Hong Kong University, Hong Kong, has also designed primers and probes for *N* and *RdRp* genes.[37] The Department of Virology III, National Institute of Infectious Diseases, Japan, and the Department of Medical Sciences, Ministry of Public Health, Thailand, have also designed primers and probes for the *N* gene.[38,39]. The CDC, Atlanta, GA, USA, has designed primers and probes for *RdRp*, *N1*, *N2*, and *N3* genes.[40] In Germany, primers and probes were designed for *E*, *N*, and *RdRp* genes.[41]

Findings of the present study

The collected data from different studies were further analyzed to determine the sensitivity level among different specimens. The highest detection rate was found in bronchoalveolar lavage fluid (98.3%), followed by saliva (91.7%), respiratory swabs (77.9%), sputum (75.8%), stool (54%), fibrobronchoscope brush biopsy (46.2%), respiratory swabs (40.3%), serum (39.4%), blood (1%), and urine (0.73%). In contrast, among the respiratory swabs, the highest detection rate was determined in nasal swabs (77.9%), followed by throat swabs (56.8%), nasopharyngeal swabs (39.6%), oropharyngeal swabs (35.7%), and pharyngeal swabs (24.8%) as shown in Tables 4 and 5.

Recommendations and conclusion

COVID-19 is still spreading worldwide with increasing number of cases and deaths. It is also understood that bats are the primary source of transmission, as with SARS and MERS. The incubation period of COVID-19 is slightly higher than that of SARS and MERS. In terms of clinical manifestation, imaging technology, and RT-PCR, the laboratory specimens and diagnostic procedures of COVID-19 are similar to those of SARS and MERS.

The rapid identification of SARS-CoV-2 was only possible due to modern sequencing technology along with lessons learned from previous coronavirus pandemics (SARS and MERS). Thanks to modern technology, primers and probes were designed in different countries within several weeks of outbreak identification and are now used worldwide. These technologies assist in the detection and diagnosis of patients with COVID-19. Different specimen types are used for RT-PCR, with the highest sensitivity being found in bronchoalveolar lavage fluid, saliva, nasal swabs, and sputum. In contrast, the lowest sensitivity was noted in urine and blood. Hence, we recommend the use of bronchoalveolar

Table 3 Detection of COVID-19 by RT-PCR in various clinical specimens collected by different researchers.

Author	Study country	Specimen	Total sample size	SARS-CoV-2 positive	References
Zhang et al.	China	Stool	83	54.2 (45)	42
		Urine	65	1.5 (01)	
		Serum	94	39.4 (37)	
		Respiratory swab	96	100 (96)	
Zheng et al.	China	Oropharyngeal swabs	14	35.7 (05)	43
		Stool	14	35.7 (05)	
To et al.	Hong Kong	Saliva	12	91.7 (11)	44
Lin et al.	China	Sputum	52	76.9 (40)	23
		Throat swab	52	44.2 (23)	
Yang et al.	China	Sputum	142	88.7 (126)	45
		Nasal swab	490	73.3 (359)	
		Throat swab	205	60.0 (123)	
		Bronchoalveolar lavage fluid	29	100 (29)	
Wang et al.	China	Bronchoalveolar lavage fluid	15	93.0 (14)	32
		Fibrobronchoscope brush biopsy	13	46.0 (06)	
		Sputum	104	72.0 (75)	
		Nasal swab	08	63.0 (05)	
		Pharyngeal swabs	398	32.0 (126)	
		Feces	153	29 (44)	
		Blood	307	1.0 (03)	
		Urine	72	0 (0)	
Liu et al.	China	Bronchoalveolar lavage fluid	05	100 (05)	46
		Nasal and pharyngeal swabs	4818	38.3 (1910)	
		Sputum	57	49.1 (28)	
Ren et al.	China	Pharyngeal swabs	1415	44.0 (623)	47
		Stool	259	69.9 (181)	

Table 4 Comparison of RT-PCR positivity in various specimens collected from different studies.

Specimen	Total sample size	Positive	Percentage (%)
Bronchoalveolar lavage fluid	59	58	98.3
Saliva	12	11	91.7
Sputum	355	269	75.8
Stool	509	275	54.0
Fibrobronchoscope brush biopsy	13	06	46.2
Respiratory swabs	7496	3018	40.3
Serum	94	37	39.4
Blood	307	03	1.0
Urine	137	01	0.73
Total	**8982**	**3678**	**40.9**

RT-PCR: reverse transcription polymerase chain reaction.

lavage fluid, saliva, nasal swabs, and sputum for the detection of SARS-CoV-2, as opposed to urine, serum, and blood.

Serological tests with high specificity and sensitivity are necessary for the rapid diagnosis of COVID-19. The development of point-of-care testing (POCT) may further improve the diagnostic capacities of laboratories. Sensitivity and specificity must be compared among serological testing, RT-PCR, POCT, and other available assays to improve diagnostics.

Table 5 SARS-CoV-2 detection in various respiratory samples via RT-PCR.

Respiratory swab	Total sample size	Positive	Percentage (%)
Nasal swab	594	463	77.9
Throat swab	257	146	56.8
Nasal and pharyngeal swabs	4818	1910	39.6
Oropharyngeal swabs	14	50	35.7
Pharyngeal swabs	1813	449	24.8
Total	7496	3018	40.3

RT-PCR: reverse transcription polymerase chain reaction.

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