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Review

Current diagnostic approaches to detect two important betacoronaviruses: Middle East respiratory syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

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ABSTRACT

Middle East respiratory syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are two common betacoronaviruses, which are still causing transmission among the human population worldwide. The major difference between the two coronaviruses is that MERS-CoV is now causing sporadic transmission worldwide, whereas SARS-CoV-2 is causing a pandemic outbreak globally. Currently, different guidelines and reports have highlighted several diagnostic methods and approaches which could be used to screen and confirm MERS-CoV and SARS-CoV-2 infections. These methods include clinical evaluation, laboratory diagnosis (nucleic acid-based test, protein-based test, or viral culture), and radiological diagnosis. With the presence of these different diagnostic approaches, it could cause a dilemma to the clinicians and diagnostic laboratories in selecting the best diagnostic strategies to confirm MERS-CoV and SARS-CoV-2 infections. Therefore, this review aims to provide an up-to-date comparison of the advantages and limitations of different diagnostic approaches in detecting MERS-CoV and SARS-CoV-2 infections. This review could provide insights for clinicians and scientists in detecting MERS-CoV and SARS-CoV-2 infections to help combat the transmission of these coronaviruses.

Abbreviations: acpcPNA, pyrrolidinyl peptide nucleic acid; ALI, Acute lung injury; ASO, Antisense oligonucleotides; BSL, Biosafety level; CDC, Centers for Disease Control and Prevention; CoV, Coronavirus; COVID-19, Coronavirus disease of 2019; CRISPR, Clustered regularly interspaced short palindromic repeats; CT, Computed tomography; CXR, Chest radiography; ddNTPs, dideoxynucleotide triphosphates; DNA, Deoxyribonucleic acid; dPCR, Digital polymerase chain reaction; EIA, Enzyme immunoassay; ELISA, Enzyme-linked immunosorbent assay; EUA, Emergency use authorization; FDA, Food and Drug Administration; FIPV, Feline infectious peritonitis virus; Hel, Helicase; HIV, Human immunodeficiency virus; ICU, Intensive care unit; IFA, Immunofluorescence assay; LAMP, Loop-mediated isothermal amplification; LOD, Limit of detection; LOQ, Limit of quantification; MALDI-TOF, MassARRAY matrix-assisted laser desorption ionization-time of flight; MERS, Middle East respiratory syndrome; MN, Microneutralisation; MS, Mass spectrometry; NP, Nucleocapsid protein; NSP, Non-structural proteins; ORF, Open reading frame; RAD, Rapid antigen test; PCR, Polymerase chain reaction; PFU, Plaque forming units; POCT, Point of care testing; ppNT, Pseudoparticle neutralization test; PRNT, Plaque reduction neutralization test; qPCR, Quantitative or real-time polymerase chain reaction; qRT-PCR, Real-time reverse transcription polymerase chain reaction; RBD, Receptor binding domain; RdRp, RNA-dependent RNA polymerase; RFLP, Restriction fragment length polymorphism; RNA, Ribonucleic acid; RSV, Respiratory syncytial virus; RT-iiPCR, Reverse transcription-insulated isothermal polymerase chain reaction; RT-RPA, Reverse transcription recombinase polymerase amplification; SARS, Severe acute respiratory syndrome; SNP, Single nucleotide polymorphism; TCID, Tissue culture infectious dose; TRPMSS2, Type II transmembrane serine protease; upE, Regions upstream of gene E; VOC, Variants of concern; VOHC, Variant of high consequence; VOI, Variant of interest; VUM, Variant under monitoring; WGS, Whole-genome sequencing; WHO, World Health Organization; OSN-qRT-PCR, One-step single-tube nested (OSN)-qRT-PCR; 5'-UTR, 5'-untranslated region.

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1. Introduction

Human coronavirus was first discovered in the 1960s and it is characterized as a virus that is responsible for causing respiratory tract infections in the human population [1]. Since the year 2003, at least six different types of coronaviruses have been discovered and these include severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1–3]. The two coronaviruses which have caused massive transmission in the human population in the past ten years are MERS-CoV and SARS-CoV-2 [3,4].

MERS-CoV infection was initially reported in the year 2012 in the Kingdom of Saudi Arabia [5], and till the year 2020, more than 2,500 MERS-CoV cases have been reported worldwide and more than 880 patients had passed away because of this coronavirus infection [3,6]. The fatality rate of MERS-CoV infection is about 35% [6,7], which is way higher than the SARS outbreak that happened in the year 2003, in which the fatality rate of SARS-CoV infection was around 11% [3]. The MERS-CoV outbreak peaked and declined in four years' time, from the year 2012–2015 [8]. Regardless, the transmission of this coronavirus still happens in a sporadic pattern and the last case was reported in December 2020 [6]. Compared to the MERS-CoV outbreak, the SARS-CoV epidemic was peaked in the year 2002–2003 and the transmission of this coronavirus has stopped since then [3,9].

SARS-CoV-2 was first reported in the Hubei province, China, at the end of the year 2019, and was declared a global pandemic crisis in early 2020 [4]. SARS-CoV-2 is responsible for causing coronavirus disease 2019 (COVID-19) [10]. Until today (17th July 2021), more than 180 million Covid-19 cases have been reported worldwide and more than four million people have passed away because of Covid-19 [11]. Even though SARS-CoV-2 is causing more massive transmission than SARS-CoV and MERS-CoV, the fatality rate of SARS-CoV-2 infection is estimated to be around 2–3% [12], much lower than that of both the SARS-CoV [3] and MERS-CoV [6].

One of the biggest challenges in detecting MERS-CoV and SARS-CoV-2 infections is that these coronaviruses would cause respiratory tract infections similar to bacteria and other common cold viruses such as influenza virus and rhinovirus [13,14]. Therefore, it would be difficult to detect the presence of coronavirus infection in patients who are presented with common cold symptoms such as fever, cough, and sore throat [14]. Besides, MERS-CoV and SARS-CoV-2 are highly pathogenic coronaviruses that can cause acute lung injury (ALI), severe pneumonia, and severe acute respiratory distress syndrome (SARS) which could be life-threatening [13]. A previously published report [15] described that the impact which could be brought by coronavirus infection "is more than a common cold infection" as it has the potential to cause a global outbreak that is accompanied by high mortality and morbidity rate.

To date, numerous studies and guidelines have outlined and compared different diagnostic approaches to detect MERS-CoV and SARS-CoV infections, which include clinical assessment, molecular diagnosis, serological diagnosis, and radiological diagnostic approaches [16–18]. With the presence of multiple, different diagnostic approaches to confirm the presence of MERS-CoV and SARS-CoV infections, it could be confusing sometimes for the clinicians and diagnostic laboratories to decide the best strategies to detect MERS-CoV and SARS-CoV infections [17,18]. Besides, it is also not easy to differentiate MERS-CoV and SARS-CoV-2 infections from each other, and from other common cold virus infections [3,13,14]. This review, therefore, aims to provide an up-to-date comparison of the advantages and limitations of different diagnostic strategies in detecting MERS-CoV and SARS-CoV infections. It is hoped that this review would provide fruitful insights for the clinicians and scientists in detecting and confirming MERS-CoV and SARS-CoV-2 infections to help fight against these coronaviruses.

2. Brief overview of MERS-CoV and SARS-CoV-2

Coronavirus is a type of enveloped, positive-sense, single-stranded RNA virus grouped under the *Coronaviridae* family [19]. This virus has the potential to infect vertebrates such as humans and animals and is divided into four different types: alpha, beta, gamma, and delta coronavirus [20]. Alpha and beta coronaviruses are by far the only viruses that could infect mammals, while gamma and delta coronaviruses only infect birds [20]. MERS-CoV and SARS-CoV are classified as betacoronaviruses in which MERS-CoV is believed to originate from the bat and was spread to humans via camel [3]. The origin of SARS-CoV-2 is still in debate and it is hypothesized that this virus was originated from bats, since there is no sufficient evidence to suggest that it is an intentionally engineered novel coronavirus [21].

Compared to other RNA viruses, coronavirus has the biggest RNA viral genome which ranges from 26,000 bp to 32,000 bp in length [19,22]. It was reported that the sequence identity between SARS-CoV, SARS-CoV-2, and MERS-CoV is at least 80% [19]. However, in another report, it was said that SARS-CoV and SARS-CoV-2 share around 80% of the sequence similarity while the sequences between SARS-CoV-2 and MERS-CoV are only about 50% similar [23]. The International Committee on Taxonomy of Viruses (ICTV) recommends that viruses sharing more than 90% of sequence identity in the conserved replicase domains belong to the same species [24]. Thus, it was concluded that both MERS-CoV and SARS-CoV-2 are novel and distinct betacoronavirus [5,23]. The genome of MERS-CoV contains around 30,110 nucleotides [19], and this genome size is slightly larger compared to the genome of SARS-CoV-2 which contains about 30,000 nucleotides [19,25]. In terms of infectivity, SARS-CoV-2 was reported to be more infectious as compared to both SARS-CoV and MERS-CoV [19]. One of the possible reasons to explain this phenomenon is that some mammals could potentially act as intermediate hosts during SARS-CoV-2 transmission and the virus is believed to acquire a certain level of mutation in order for them to transmit to the human from the intermittent host [19].

The RNAs of both viruses are capped at the 5' end and poly-A tail is found at their 3' end [19,22]. As SARS-CoV, MERS-CoV and SARS-CoV-2 have a certain level of similar sequence identity as reported previously [19], therefore, the genetic structures of these three viruses are also quite similar. Generally, the viral genomes contain open reading frame (ORF) 1a and 1b, *spike* (S) gene, *envelope* (E) gene, *membrane* (M) gene, and *nucleocapsid* (N) gene [18,19,22]. According to the 5' to 3' order of arrangement, ORF1a and ORF1b are situated towards the 5' end, followed by S, E, M, and N genes [18,19,22]. All these genes play vital roles in virus virulence, virus entry, and survival in the targeted host cells [18,19,22]. The S gene encodes for spike protein which helps facilitate the binding and attachment of the virus to the host cell [26], whereas the M gene encodes M protein which maintains the virus shape and structure [22]. E gene encodes E protein that plays an essential role in viral assembly and production [22]. N protein coded by the N gene is important in binding to the viral RNA and this protein helps in regulating viral replication [19,22]. Other than structural protein-encoding genes, both MERS-CoV and SARS-CoV-2 also carry genes that encode a number of non-structural proteins (NSPs) that are important in regulating various virus activities such as viral replication [3,19,22,27].

3. Different diagnostic approaches to detect MERS-CoV and SARS-CoV-2 infections

To date, there are several published methods and approaches (Fig. 1) which could be used to screen and diagnose MERS-CoV and SARS-CoV-2 infections. These methods can be generally divided into three main categories, which refer to clinical diagnosis, laboratory diagnosis, and radiological diagnosis (Table 1) [16–18]. Laboratory diagnosis can be further sub-divided into nucleic acid-based detection, protein-based detection, or viral culture to confirm the virus infections [16–18,28].

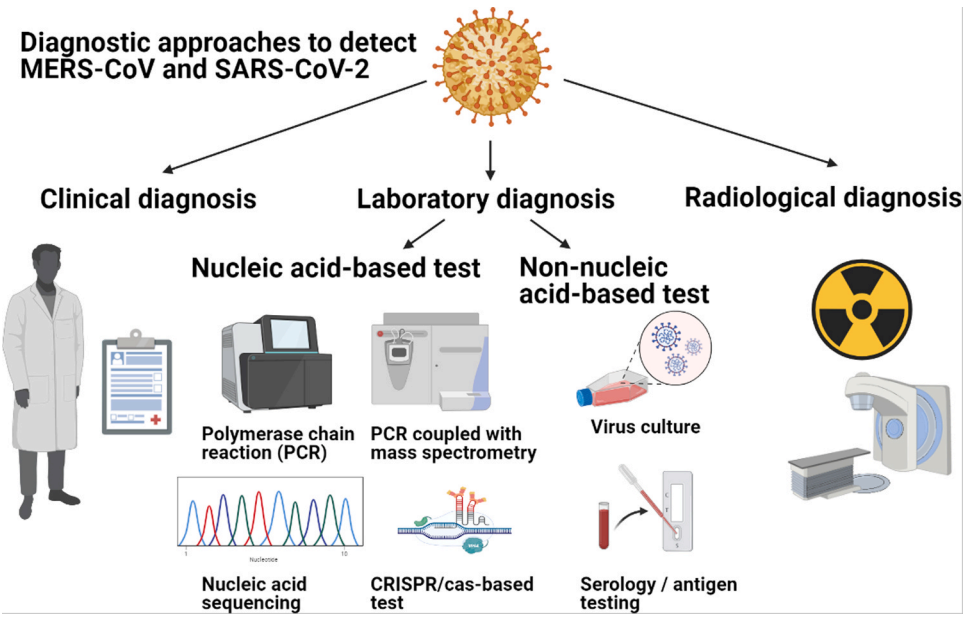

Fig. 1. Various diagnostic approaches which could be used to detect MERS-CoV and SARS-CoV infections. Clinical diagnosis relies on history taking and clinical assessment to determine whether an individual is at high risk of contracting the coronaviruses [6,29,30,33]. Laboratory diagnostic tests can be divided into nucleic acid-based, protein-based, and virus-culture tests [16,17]. Examples of nucleic acid-based diagnostic tests include polymerase chain reactions like qRT-PCR and dPCR, or RT-LAMP, RT-RPA, PCR-coupled with mass spectrometry (MS) and CRISPR/Cas-based detection test [16,17,99,104,105,118,121,126,185,54,57,66,74,77,95–97]. Protein-based diagnostic tests include virus serology, neutralization and virus antigen tests [2,16,17,54,57,126,180,185,257]. Radiological diagnosis involves the use of chest radiography or CT scan to assess the thoracic cavity of the individuals who are suspected to have pneumonia secondary to coronavirus infection [16,277–279].

Table 1
Overview and comparison of the strengths and limitations of different diagnostic approaches to detect MERS-CoV and SARS-CoV-2.

	Clinical diagnosis	Laboratory diagnosis							Radiological diagnosis
		Nucleic acid-based tests					Non-nucleic acid-based tests		
		PCR-based methods	Isothermal amplification-based detection methods	Nucleic acid sequencing	CRISPR/Cas-based method	Others: Mass spectrometry detection approach	Virus culture	Protein-based tests	
Description	Assessment on the patient history and clinical features	Quantitative and qualitative detection of virus	Quantitative and qualitative detection of virus	Virus genome sequencing	Detection of specific viral genetic regions	MS approach to detect viral genetic targets	Live culture of virus	Antibody or antigen detection of the viral proteins	Radiological evaluation of the thoracic cavity of the individual
Advantages	Fast	Gold standard test, highly sensitive and specific	Might require shorter test time than qRT-PCR and do not need sophisticated instruments	Alternative test to detect the virus, differentiating different coronaviruses	Might require shorter test time	Highly sensitive and specific, faster test results	Could be used in vaccine development	Fast	Non-invasive
Limitations	Only clinicians could assess, less sensitive than molecular tests	Require specialized machine and trained staff to perform	Might be less sensitive and specific than qRT-PCR	Require specialized machine and trained staff to perform, expensive	Require trained staff to perform, expensive, multi-steps reactions might cause contamination	Require specialized machine and trained staff to perform, expensive	Require trained staff to perform, time-consuming	Risk of cross-reactivity with other pathogens	Require radiological facilities to perform the test, less sensitive than molecular tests
References	[29–33]	[16,17,54,57, 60,66,67]	[95–97,104, 105,116,118, 121]	[29,34,54,57, 126]	[16,144,145, 148,149]	[156,157]	[16,32,35, 116,118]	[26,54,57, 88,107, 108,110, 111,132]	[16,17, 277–279]

3.1. Clinical diagnosis of MERS-CoV and SARS-CoV-2 infections

Assessing the patient history like a history of travel to coronavirus outbreak area and history of having close contact with confirmed cases are helpful to screen whether the patient is having a high risk to contract coronavirus [29,30]. Besides, clinical evaluation of signs and symptoms which could be caused by MERS-CoV and SARS-CoV-2 infections is useful to rapidly screen for suspected cases at the triage area of the health facility [29,31]. However, like other microorganisms or common cold viruses which could cause respiratory tract infections, both MERS-CoV and SARS-CoV-2 infections would also cause several common clinical signs and symptoms suggestive of respiratory system infection, such as cough, shortness of breath, respiratory distress, rhinorrhea, and hemoptysis [5,29,30,32]. Besides, the coronavirus infections were also reported to show clinical symptoms in the gastrointestinal tract (vomiting and diarrhea), neurological system (headache and confusion), musculoskeletal system (muscle pain), and other general symptoms including fever [5,9,27,29,30,32]. As most of these clinical signs and symptoms are non-specific to coronavirus infection, clinical evaluation alone could not be used to confirm the coronavirus infection [29,30,33,34]. In addition, some of the patients infected with MERS-CoV and SARS-CoV-2 might be asymptomatic. Therefore, all suspected individuals who have recent contact with the confirmed MERS-CoV and SARS-CoV-2 patients, or had a history of

travel to the outbreak areas, should be subjected to further laboratory testing to confirm the diagnosis of MERS-CoV and SARS-CoV-2 infections [29,30,33,34].

In severe cases of MERS-CoV and SARS-CoV-2 infections, the patients might be presented with severe pneumonia, respiratory failure, or severe acute respiratory syndrome, in which these patients need to be supported in the intensive care unit (ICU) with mechanical ventilation support [12,13,35]. Regardless, none of these symptoms are exclusive to MERS-CoV and SARS-CoV-2 infections, therefore could not be used to differentiate MERS-CoV and SARS-CoV-2 infections from infections caused by other causative agents such as a respiratory syncytial virus (RSV) [36,37].

3.2. Laboratory detection of MERS-CoV and SARS-CoV-2 infections

Generally, laboratory tests that could be employed to detect MERS-CoV and SARS-CoV-2 can be divided into the nucleic acid-based test, protein-based test, or viral culture, and each of these tests has its own advantages and limitations (Table 1). Nucleic acid-based detection methods which have been reported in detecting these coronaviruses include polymerase chain reaction (PCR), loop-mediated isothermal amplification methods (LAMP), next-generation sequencing (NGS), CRISPR/Cas-based detection test, and some other tests [16–18,28]. Protein-based tests which could be used to detect MERS-CoV and SARS-CoV-2 infections comprise viral serology and antigen tests (Fig. 1) [16–18,28].

3.2.1. Nucleic acid-based detection of MERS-CoV and SARS-CoV-2

Molecular diagnosis is important in the identification, prevention, and treatment of infectious diseases [38]. Molecular diagnosis is a rapidly growing discipline in laboratory medicine, with new methods and applications continually becoming available and improvements being made [38,39]. Viral nucleic acids can be detected using molecular approaches such as polymerase chain reaction (PCR), reverse transcription loop-mediated isothermal amplification (RT-LAMP), and next-generation sequencing (NGS), and these methods are making their way into clinical laboratories [16,18,40]. Molecular tests enable the rapid detection of unculturable or fastidious microorganisms from clinical samples without the need for culture [41]. In addition, sequence analysis of amplified DNA from the infectious agents allows for identification and better characterization of the virus [42]. Besides, sequence analysis allows scientists to correlate the relationships of different pathogens based on their phylogenetic relationships [42]. The variation in the viral subspecies may affect infectivity, thus influence the disease prognosis [43]. Viral genome sequencing also enables the direct detection of genes or gene mutations in the virus which are responsible for contributing to its virulence and drug resistance [44]. On top of that, the molecular diagnostic technique is useful to quantify viral load [45]. With the advancement in molecular diagnostic technology, the presence of computerized, automated machines and handy software has allowed molecular diagnosis to be conducted more commonly with high precision [46]. In short, the detection of viral agents up to the nucleic acid level signifies a notable breakthrough in clinical microbiology.

3.2.1.1. Real-time reverse transcription PCR (qRT-PCR). The nature and sequence of the first MERS-CoV isolate were uncovered using a random-amplification deep-sequencing approach [5]. Following that, in the year 2013, the first MERS-CoV isolate was patented, and many authorities were concerned such an act might restrict the progress on the development of viral diagnostics tests, viral vaccines, and anti-viral drugs [47]. Fortunately, the designated authorities granted virus isolate access to the related parties as long as the applicants follow the strict listed biosafety rules [47]. The molecular detection of MERS-CoV RNA started with the sequencing of conserved domains ORF 1a and 1b [48,49]. Both ORFs help to identify the coronavirus species [48]. Thereafter, a sensitive molecular diagnostic approach using real-time reverse transcription PCR (qRT-PCR) was quickly defined, validated, and widely employed to diagnose MERS-CoV infection [27]. For SARS-CoV-2, the virus was first isolated from human airway epithelial cells from patients in Wuhan, China, at the end of the year 2019, and was subsequently subjected to genetic sequencing [50]. As SARS-CoV-2 was started to cause a global pandemic crisis in early 2020, many countries were then working hard to produce and validate different qRT-PCR diagnostic tests to detect the patients who carry the virus as part of the efforts to combat the transmission of SARS-CoV-2 [16].

Real-time PCR-based analyses combine the traditional PCR approaches and fluorescent-emitting compounds to measure the number of amplicons produced during the PCR amplification process in "real-time" [51]. By combining both real-time PCR and reverse transcription reaction, the gene expression level in the samples can be calculated [51]. With the introduction of qRT-PCR to detect both MERS-CoV and SARS-CoV, different health agencies and authorities like World Health Organization, US Food and Drug Administration (FDA), and US Centers for Disease Control and Prevention has sanctioned and revised the confirmed MERS and SARS-CoV-2 case definition in which individual who is tested positive for MERS-CoV or SARS-CoV-2 using qRT-PCR or serology test will be regarded as confirmed case, regardless of appearant clinical signs and symptoms (Fig. 2) [16,29,30,34,52–54]. Subsequently, several MERS-CoV and SARS-CoV-2 diagnostic kits which consist of the assay signatures like ORF 1a, upE, and, S, N/ RdRp, and an appropriate positive control were developed [16,18,55,56]. CDC and WHO suggest that a patient is potentially negative for active MERS-CoV infection following one negative qRT-PCR test on the tested viral genetic target as described (Fig. 2), but further testing is recommended to confirm the absence of MERS-CoV infection [29,54]. Whereas, a patient diagnosed with MERS is required to have at least two consecutive negative qRT-PCR tests on all specimens to be considered as cleared for MERS-CoV infection [54]. For SARS-CoV-2, it is also recommended that patients who are symptomatic or had a history of travel to outbreak areas or had a history of close contact with the confirmed cases be subjected to a second qRT-PCR test if their first test result is negative [30,57]. However, to discharge a patient who is confirmed to have SARS-CoV-2 infection, WHO has released updated guidelines which state that symptomatic patients could be discharged 10 days after the onset of symptoms while asymptomatic patients could be discharged 10 days after the molecular diagnosis of SARS-CoV-2 [58]. For both MERS-CoV and SARS-CoV-2, both CDC and WHO recommend that multiple specimens should be collected for molecular testings from various body sites like upper (nasopharyngeal and oropharyngeal swabs) and lower (bronchoalveolar lavage, sputum, and trachea) respiratory tracts, blood, and lower gastrointestinal tract (stool specimen) [29,54,57]. Nonetheless, numerous factors which might affect the rate of success and accuracy of qRT-PCR testing of coronavirus have been identified and these factors include type and quality of specimens, the expertise of laboratory staff, and laboratory environment (contamination) [26].

It has been recommended by WHO that qRT-PCR targeting both the upE and ORF 1a regions are highly sensitive to be used for the detection of MERS-CoV [59,60]. A study had previously reported the use of a qRT-PCR test that was aimed to target the upE and ORF 1a regions of MERS-CoV and the test was able to detect 5 RNA copies per reaction with 100% specificity (Table 2) [59]. A commercial test kit that could detect both UpE and ORF 1a regions of MERS-CoV was reported to have a sensitivity of 95% and specificity of 100% [61]. This suggested that targeting both UpE and ORF 1a could be useful to confirm the presence of MERS-CoV. Two other studies had reported that targeting UpE could be useful to specifically detect MERS-CoV infection in which the qPCR test sensitivity could be above 95% [62] and the limit of detection of such test could be 5–10 RNA copies per reaction [52]. On the other hand, another two studies reported the use of other targets like ORF 1b to confirm the presence of MERS-CoV and such qPCR tests could detect

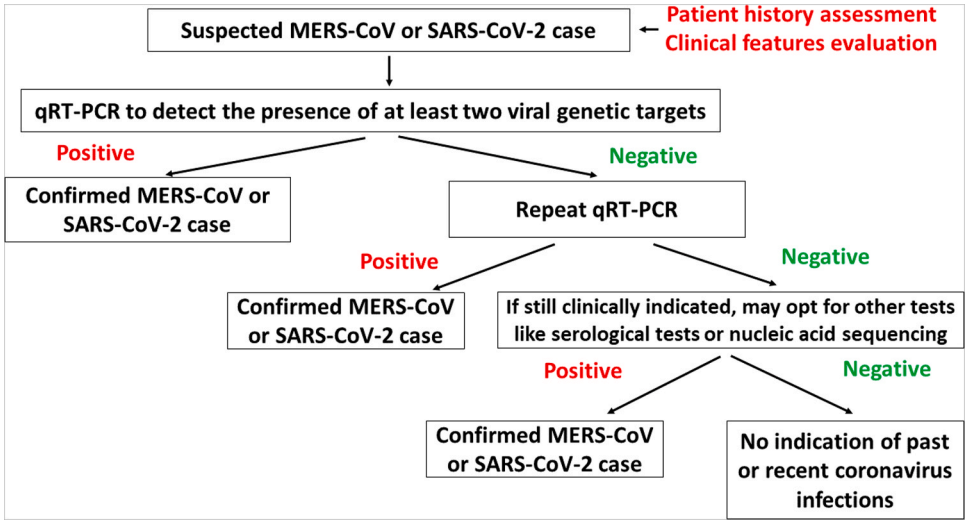

Fig. 2. Diagnostic approaches to confirm the presence of MERS-CoV and SARS-CoV-2. For clinically suspicious cases, qRT-PCR remains as the gold standard molecular diagnostic test to confirm MERS-CoV and SARS-CoV infections [6,29,30,33,34,57,126]. For individuals who have negative qRT-PCR test results, a repeat test is highly recommended [29,34,54,57]. In the case in which qRT-PCR results are inconclusive, further tests like nucleic acid sequencing and serological tests could be performed to confirm the coronavirus infection [29,34,54,57]. For MERS-CoV, the guidelines state that the patients need to have at least two consecutive negative qRT-PCR results before they are allowed to be discharged from the isolation services [29,54]. However, for SARS-CoV-2, the latest guideline recommends that the symptomatic patient is allowed to be discharged 10 days after the onset of symptoms while asymptomatic patients could be discharged 10 days after the molecular diagnosis of the virus. [58].

4–64 RNA copies per reaction with a specificity of 100% [55,60]. Compared to the previously mentioned genetic targets of MERS-CoV, the use of other targets like the N region might not be as sensitive as upE and ORF regions in confirming the presence of MERS-CoV. It was reported that the use of qRT-PCR to detect the N gene of MERS-CoV had a sensitivity that ranged from 55–100% and the specificity could vary from 33% to 100% [63,64]. These findings suggested that the use of N gene detection to confirm MERS-CoV infection should be accompanied by tests that target other genetic regions like upE and ORF regions to avoid false-negative results. A group of Chinese researchers had previously reported the use of leader sequence at the 5'-untranslated regions (5'-UTR) of the MERS-CoV to confirm the presence of the virus and it was demonstrated that such qRT-PCR test could detect 5 RNA copies per reaction or 5.62×10^{-2} $TCID_{50}$/mL with 100% specificity [65]. The findings from this study highlighted that targeting the 5'-UTR regions of the virus could also be useful to detect MERS-CoV [65].

Compared to MERS-CoV-2, there are more studies which had described the use of qRT-PCR and qPCR in confirming the diagnosis of SARS-CoV-2 (Table 2) [66–71]. Some studies had described the use of a single target like N gene to detect the presence of SARS-CoV-2 and such qRT-PCR test could detect around 5 RNA copies per reaction with a sensitivity of above 99% and specificity of 100% [71,72]. Another two studies reported that when qPCR was used to detect N and ORF 1ab regions, the limit of detection could range from 500 to 1000 RNA copies per mL, and the test overall sensitivity was ranged from 58% to 100% with test specificity of 100% [73,74]. However, a group of Chinese scientists had reported that the limit of detection of qRT-PCR that targets both N and ORF 1ab could be as low as 1–10 RNA copies per reaction [75] and this implied that the test sensitivities could vary between different research groups and experimental conditions [73–75]. On contrary, three research groups had employed three SARS-CoV-2 targets that include N, E, and RdRp in their qPCR testings, and the test sensitivities were varied between different published reports but the test specificities were quite consistent (100%) between the three published findings [70,72,76]. Compared to N and E genes, the detection of RdRp could be at least 20-folds less sensitive and this suggested that both N and E genes should be selected to be used in the qPCR test to confirm SARS-CoV-2 infection, followed by RdRp [70]. In terms of COVID-19 RdRp-qRT-PCR assay to detect SARS-CoV-2, a study has shown that when two different RdRp-based assays, namely RdRp/Hel and RdRp-P2 assays were used to detect SARS-CoV-2, the use of RdRp/Hel based assay was more sensitive than the other one in detecting the presence of SARS-CoV-2 [77]. In addition, the RdRp/Hel based assay was also found to have comparable sensitivity and specificity with N and S targets-based qPCR assay [77], suggesting that this RdRp/Hel based assay could be potentially used as an alternative test to detect SARS-CoV-2.

Apart from being widely reported in numerous original research articles, the use of qRT-PCR in detecting SARS-CoV-2 has also been expanded to commercial use, and to date, at least 20 different qRT-PCR commercial kits had been approved by the FDA, and examples of these kits include RealStar SARS-CoV-2 RT-PCR kit, Solaris Multiplex SARS-CoV-2 assay, SalivaDirect, ViroKey™ SARS-CoV-2 RT-PCR test and EURORealTime SARS-CoV-2 kit [68,78,79]. All these different commercial qRT-PCR or qPCR test kits could detect different molecular targets like N, E, S, RdRp, and ORF 1ab and the limit of detection of these test kits could be varied from 0.5 RNA copies per μL to 12,500 RNA copies per mL [68,78,79]. The sensitivities and specificities of these test kits generally ranged from 94% to 100%, and 90–100%, respectively [68,78,79]. The superior sensitivities and specificities of these different SARS-CoV-2 qPCR commercial test kits have helped the local health authorities to use them in detecting the virus without the need to use conventional qRT-PCR tests in which the laboratory scientists might need to undergo more tedious and lengthy steps to get the tests done [16,17,68].

3.2.1.2. Digital PCR (dPCR). Other than qPCR, another type of PCR known as digital PCR (dPCR) can also be used to detect both MERS-CoV and SARS-CoV-2 (Table 3) [66,80,81]. dPCR is a new nucleic acid amplification technology that is made commercially available since the year 2011 [82]. The main difference between dPCR and qPCR is that in dPCR, the PCR reaction is being segregated or partitioned into thousands of individual, independent reactions before the start of amplification and thus, this would give more precise data especially if the sample is present in minute quantity [82]. For MERS-CoV, a study has shown that the use of dPCR could detect 64–167 copies of MERS-CoV virus per reaction when primers or probes that targeted N, E, and ORF 1ab were used and the dPCR specificity was 100% [81]. Compared to the qRT-PCR test that targeted similar targets that had been reported to have lower LOD (<10 RNA copies per reaction) [52,55,59,60], the sensitivity of dPCR in detecting MERS-CoV seems to be lower but the specificity of dPCR and qPCR are about the same.

Like qPCR, there were more reported studies (Table 3) that had described the use of dPCR in detecting SARS-CoV-2 than MERS-CoV [66,83–86]. When genetic targets like N, E, RdRp, and ORF 1ab were used in the SARS-CoV-2 dPCR test, the sensitivity of such tests was found to be ranging from 60% to 99% with the limit of detection that varied from 10

Table 2
Comparison of the sensitivity and specificity of qRT-PCR or qPCR in detecting different molecular targets of MERS-CoV and SARS-CoV-2.

Virus type	Molecular targets	Sensitivity (SN)	Specificity (SP)	References
MERS-CoV	N	54.5%	33%	[64]
	N, E	60–100%	100%	[63]
	N & upE	LOD: 5–10 RNA copies/reactions	100%	[52]
	upE, ORF 1a	LOD: 0.5–0.9 RNA copies/μL (~95%)	100%	[61]
	upE & ORF 1a	LOD: ~5 RNA copies/reaction for singleplex and multiplex qRT-PCR	100%	[59]
	upE & ORF 1b	upE: LOD of 3.4 RNA copies/reaction ORF 1b: LOD of 64 RNA copies/reaction	100%	[60]
	UpE, S	>98%	Unclear	[62]
	ORF 1b	ORF 1b: LOD of 4.1 RNA copies/reaction	100%	[55]
	5'-UTR	LOD: 5 RNA copies/reaction or 5.62×10^{-2} $TCID_{50}$/mL	100%	[65]
SARS-CoV-2	N	LOD: 0.0187 ng RNA compared to LOD of dPCR of 0.00187 ng.	Unclear	[66]
	N	LOD: 5 RNA copies/reaction (>99%)	100%	[318]
	N	LOD: <5 RNA copies/reaction (100%)	100%	[71]
	N, ORF 1ab	LOD: 500–1000 RNA copies/mL (100%)	95.3%	[73]
	N, ORF 1ab	LOD: 1–10 RNA copies/reaction	100%	[75]
	N, ORF 1ab	ORF 1ab: LOD of 520.1 RNA copies/mL N: LOD of 528.1 RNA copies/mL Overall sensitivity to detect positive Covid-19 samples: 58.82%	100%	[74]
	N, E, RdRp	≥79%	100%	[76]
	N, E, RdRp	LOD: 80–154 RNA copies/mL (90–100%)	100%	[72]
	N, E, RdRp	LOD: ≥ 10 RNA copies/reaction (For N & E); LOD for RdRp could be 20-folds less sensitive	Unclear	[70]
	N, E. NSP, RdRp	LOD: 100 RNA copies/μL (~100%)	100%	[88]
	N, S, RdRp/Hel, RdRp-P2	N, S, RdRp/Hel: 1.8×100 $TCID_{50}$/mL RdRp-P2: 1.8×101 $TCID_{50}$ /mL	100%	[77]
	N, E, ORF 1ab, RdRp	E: Detectable at 1:80 dilution N: Detectable at 1:160 dilution ORF 1ab: Detectable at 1:40 dilution RdRp: Detectable at 1:10 dilution	100%	[93]
	N, E, S, ORF 1ab, RdRp	60–97.7% (depending on targets)	Unclear (However, false negative ranged from 2% to 40%)	[319]
	N, E, S, ORF 1ab, RdRp	E: LOD95 of 0.91–4.8 RNA copy/mL N: LOD95 of 4.8 RNA copy/mL S: LOD95 of 3.8–4.3 RNA copy/mL ORF 1ab/RdRp: LOD95 of 3.1–23 RNA copy/mL	100%	[67]
	N, E, S, ORF 1ab, RdRp	LOD: 0.5 RNA copies/μL to 12,500 copies/mL (94.1–100%)	90–100%	[68,78,79]

Table 3
Comparison of the sensitivity and specificity of dPCR in detecting different molecular targets of MERS-CoV and SARS-CoV-2.

Virus type	Molecular targets	Sensitivity (SN)	Specificity (SP)	References
MERS-CoV	N, E, ORF 1ab	E: LOD of 167 RNA copies/reaction N: LOD of 156 RNA copies/reaction ORF 1ab: LOD of 64 RNA copies/reaction	100%	[81]
SARS-CoV-2	N	LOD and sensitivities of both qRT-PCR and dPCR are highly correlated. Mean detectable copies/samples using both methods are unclear	Unclear	[83]
	N	LOD: 2.5 RNA copies/reaction as compared to 10 RNA copies/reaction of qRT-PCR (≥86%)	Unclear	[84]
	N	LOD: 0.00187 ng RNA compared to LOD of qRT-PCR of 0.0187 ng Could detect up to 0.08 virus copies/μL after 10-fold dilutions	100%	[66]
	N	dPCR able to distinguish true positive and negative samples with low viral load (10^{-4} dilutions) while qRT-PCR was unable	100%	[80]
	N	LOD: <2 RNA copies/μL for dPCR while qRT-PCR could not	Unclear	[86]
	RdRp	Median detection: 128 RNA copies/mL (~99%)	~95%	[87]
	N, ORF 1ab	ORF 1ab: LOD of 401.8 RNA copies/mL N: LOD of 336.8 RNA copies/mL Overall sensitivity to detect positive Covid-19 samples: 67.65%	100%	[74]

to 400 RNA copies per mL [74,85,87]. When compared to qRT-PCR tests that targeted similar targets that could achieve higher test sensitivity, for example, the sensitivity of almost 100% [71,73,88], the sensitivity of dPCR reported in these studies seems to be lower [74,85,87]. However, on the other hand, when another four studies were conducted to compare the efficiency, sensitivity, and specificity of qRT-PCR and dPCR in detecting the N gene of SARS-CoV-2, the findings showed that generally, dPCR has higher sensitivity and specificity than qRT-PCR (Table 2) [66,80,84,86]. In another word, when the SARS-CoV-2 viral load is too low to be detected by qRT-PCR, dPCR could be utilized to detect the virus [66,80,84,86]. Another Italian study showed that the LOD and sensitivities of qRT-PCR and dPCR in detecting N gene of SARS-CoV-2 are highly correlated and comparable and this further

supported that dPCR could be employed as an alternative COVID-19 diagnostic test other than qRT-PCR [83].

Apart from being utilized widely to diagnose MERS-CoV and SARS-CoV-2 infections, PCR like qRT-PCR and dPCR are also being used to monitor viral load and disease progression [66,80,89–91]. Several studies have found a direct association between high MERS-CoV loads in clinical samples and worse clinical outcomes [89,90], where the high abundance of virions could lead to increased lung damage via direct destruction of respiratory cells or initiate an exaggerated inflammatory response [92]. For SARS-CoV-2, it was proposed that high viremia is associated with a hyperinflammation state which will then lead to endothelial damage, perivascular inflammation, and systemic micro-and macrovascular complications [91]. To sum up, the introduction of different PCR methods like qRT-PCR and dPCR has helped in confirming coronavirus infection and also aided in the clinical management of the disease development.

3.2.1.3. Reverse transcription loop-mediated isothermal amplification (RT-LAMP). To date, qRT-PCR remains one of the most sensitive and specific tests to detect both MERS-CoV and SARS-CoV-2 [59,93]. However, researchers and laboratories are still looking for a simpler method that can amplify targeted viral genes with high sensitivity, specificity, and rapidity [94]. Because of this, several isothermal amplification methods have been developed to detect both MERS-CoV and SARS-CoV, which include reverse transcription loop-mediated isothermal amplification (RT-LAMP) [94,95] and reverse transcription recombinase polymerase amplification (RT-RPA) assays [48,96,97].

RT-LAMP is a rapid and simple nucleic acid amplification assay that relies on Bst DNA polymerase large fragment for target amplification and could generate approximately 10^9 DNA copies in less than an hour [98]. Compared to RT-PCR, RT-LAMP reaction occurs at lower temperature (60–65 °C) [48,98]. This means that it does not require sophisticated and expensive equipment for precise temperature control. Besides, some of the RT-LAMP assays can be completed within one hour [99] as compared to qRT-PCR which could usually take up to three to four hours to complete [100]. To detect the N gene of MERS-CoV, RT-LAMP was found to have LOD of 0.4 RNA copies per reaction in a Korean study [99] and LOD of 10–20 RNA copies per μL in a Chinese study [101], and the test specificities of both studies were 100% [99,101]. When RT-LAMP was used to detect the presence of other MERS-CoV targets like ORF 1a and ORF 1b, the test sensitivity was found to varied from 2 to 120 RNA copies per reaction in which the test specificity was also 100% (Table 4) [102,103]. In addition, the test sensitivity of RT-LAMP was proven to be equivalent to qRT-PCR [103]. A similar study which was aimed to detect upE and ORF 1a of MERS-CoV was also found that both RT-LAMP and qRT-PCR have comparable and equivalent sensitivities, further suggesting that RT-LAMP could be used as an alternative diagnostic tool other than qRT-PCR [94]. The only study which has shown that RT-LAMP was less sensitive than qRT-PCR in detecting MERS-CoV was an American study that reported that RT-LAMP could be 2 to 200-folds less sensitive than qRT-PCR in detecting ORF 1ab of MERS-CoV to confirm the presence of the virus [48].

As RT-LAMP has been proven to be a sensitive diagnostic test to detect MERS-CoV, therefore, this method was also being tested and used to confirm SARS-CoV-2 infection when this virus started to cause a global public health crisis since the end of 2019 [95,104,105]. To date, several genetic targets of SARS-CoV-2 have been reported to be able to be detected by RT-LAMP (Table 4) and one of them was the N gene [95,106]. RT-LAMP was able to detect 100 RNA copies per reaction [106] or 100 RNA copies in 1 μL of the tested sample [95]. Compared to qRT-PCR, RT-LAMP was found to have lesser sensitivity to detect N gene of SARS-CoV-2 even though both qRT-PCR and RT-LAMP could have comparable specificity (~100%) in detecting SARS-CoV-2 [95,106]. The lower sensitivity of RT-LAMP in detecting SARS-CoV-2 was further

Table 4
Comparison of the sensitivity and specificity of RT-LAMP in detecting different molecular targets of MERS-CoV and SARS-CoV-2.

Virus type	Molecular targets	Sensitivity (SN)	Specificity (SP)	References
MERS-CoV	N	LOD: 1–2 × 10^1 RNA copies/μL of samples	100%	[101]
	N	LOD: 0.4 RNA copies/reaction	100%	[99]
	ORF 1ab	0.02–0.2 plaque forming units (PFU) 2–200-folds less sensitive than qRT-PCR	100%	[48]
	N, ORF 1ab	LOD: 120 RNA copies/reaction	100%	[102]
	N, ORF 1a	LOD: 2 RNA copies/reaction (same as qRT-PCR)	100%	[103]
	upE & ORF 1a	upE: LOD of 1.6 RNA copies/reaction ORF 1a: LOD of 3.4 RNA copies/reaction *Equivalent sensitivity to qRT-PCR	~100%	[94]
SARS-CoV-2	N	LOD: 100 RNA copies/reaction (10-folds lesser than qRT-PCR)	100%	[106]
	N	LOD: ~ 100 RNA copies/μL sample 86% (poorer than qRT-PCR)	99.5% (poorer than qRT-PCR)	[95]
	N	LOD: 900 RNA copies/mL (+ve agreement: 94, -ve agreement: 98%)	100%	[112]
	N	LOD: 20,000 RNA copies/mL (95%)	100%	[114]
	ORF 1ab	LOD: 125 genomic equivalents/swab (96.6%)	100%	[320]
	N, E	LOD: 25–75 RNA copies/mL (+ve agreement: 98%, –ve agreement: 100%)	Cross-react with SARS-CoV	[111]
	N, ORF 1ab	LOD: 2000 RNA copies/swab (100%)	99%	[321]
	N, ORF 1ab	LOD: 1 RNA copy/μL	Unclear	[113]
	N, ORF 8	LOD: 100 RNA copies/μL (at least 2-folds lower than qRT-PCR	100%	[108]
	N, E, ORF 1ab	LOD: 0.75 RNA copies/μL (100%)	100%	[322]
	N, S, ORF 1ab	LOD: 80 RNA copies/mL (Comparable sensitivities with qRT-PCR)	100%	[104]
	N, E, RdRp, NSP	LOD: ~3 RNA copies/25 μL (44.8–82.8%)	<100% (few cross-reactivity with other pathogens)	[107]
	N, S, ORF 1a, ORF 8	LOD: 0.75 RNA copies/μL (100%)	100%	[109]
	N, S, NSP, ORF8	LOD: ~ 100 RNA copies/reaction	100%	[105]
	Unclear	LOD: 500 RNA copies/reaction	Unclear	[115]
	Unclear	LOD: 6.7 RNA copies/reaction (at Day 9) (92.8% compared to qRT-PCR)	100%	[110]

shown in a study in which the authors reported that RT-LAMP has a sensitivity that ranged from 45% to 83% and specificity of less than 100% because of the possibility of the test to cross-react with other pathogens [107]. Besides, another study also reported that the sensitivity of RT-LAMP was at least 2-folds less than qRT-PCR in detecting genetic targets of SARS-CoV-2 like N and ORF 8 genes [108]. However, in a Chinese study [104], it was demonstrated that RT-LAMP could detect 80 RNA copies per mL when targets like N, S, and ORF 1ab regions were used in the assay and the test sensitivity was comparable to that of qRT-PCR, and the RT-LAMP assay specificity was 100%. This finding was against the four mentioned studies that had proposed that RT-LAMP was less sensitive than qRT-PCR in detecting SARS-CoV [95,104,106,107]. An American study also reported that RT-LAMP could produce 100% sensitivity and specificity when genetic targets of SARS-CoV-2 like N, S, ORF 1a, and ORF 8 were targeted in the assay and the LOD of the assay could be as low as 0.75 RNA copies per μL [109]. Therefore, it could be summed up that compared to qRT-PCR, RT-LAMP could sometimes produce comparable sensitivity and specificity in detecting SARS-CoV-2 and so, it could serve as a potential diagnostic test for this viral infection when qRT-PCR results are inconclusive [95]. In terms of molecular targets, it seems like the sensitivity of RT-LAMP in detecting different molecular targets of SARS-CoV-2 could vary from one published report to the other [95,104,105,107,110], and thus, no target is said to be more reliable to be chosen as a molecular target for RT-LAMP.

As multiple studies have reported that RT-LAMP could have promising sensitivity and specificity in detecting SARS-CoV-2 [104,105,109], therefore, several developed RT-LAMP test kits were also being introduced to help diagnose COVID-19 across the world. Examples of such kits include MobileDetect BioBCC19 test kit, Lucira COVID-19 All-In-One test kit, AQ-TOP COVID-19 rapid detection kit plus, and SARS-CoV-2 RNA DETECTR assay [68,111–114]. All these RT-LAMP-based test kits were invented to detect various targets of SARS-CoV-2 like N, E, and ORF 1ab, and the sensitivity and specificity of these kits were all above 90% [68,111–115]. This means that most of these test kits are specific enough to detect SARS-CoV-2 without cross-reacting with other pathogens from the respiratory tracts or other coronavirus species [68,111–115].

3.2.1.4. Reverse transcription recombinase polymerase amplification (RT-RPA) and other isothermal amplification-based test. Another type of isothermal amplification-based assay which could be used to detect both MERS-CoV and SARS-CoV-2 is RT-RPA (Table 5) [96,97,116]. Compared to qRT-PCR, RT-RPA assay could run at a lower temperature like 42 °C for 15 min (shorter test duration) and it can amplify the specific genetic targets after the enzyme recombinase and the oligonucleotide primers bind to the specific genetic regions [96,116]. When RT-RPA was used to detect the N gene of MERS-CoV, it was shown that RT-RPA could detect as low as 1.2 RNA copies in 1 μL of a sample with 100% sensitivity [117] or 10 RNA molecules per reaction with comparable sensitivity to qRT-PCR [96]. Besides, RT-RPA was demonstrated to have 100% specificity in detecting MER-CoV [96,117]. These findings suggested that RT-RPA could serve as a sensitive diagnostic tool to detect MERS-CoV by targeting the N gene [96,117]. Compared to the N gene, RT-RPA was found to be slightly less sensitive to detect other molecular targets of MERS-CoV like UpE and ORF 1a [96,117,118]. The sensitivity of RT-RPA to detect UpE was ranged from 86% to 98.06% while the sensitivity of RT-RPA to detect ORF 1a was 99% [117,118].

For SARS-CoV-2, RT-RPA has also been widely reported to detect genetic targets of the virus such as the N gene (Table 5) and the LOD of the assay could be 5 RNA copies in 1 μL of a sample with a sensitivity of 98% [119] or 7.8 RNA molecules per reaction with 100 sensitivity as compared to qRT-PCR [120]. Besides, the assay specificity could be as high as 100% [119,120]. On the other hand, when other molecular targets like RdRp and ORF 1ab were used in RT-RPA to detect the presence of SARS-CoV-2, the assay sensitivity was lower [121,122] and the LOD of the assay could be 10-folds less sensitive than qRT-PCR [122]. These findings implied that compared to other molecular targets, the N gene seems to be the target that is easier to be detected using the RT-RPA method [120,123]. When compared to qRT-PCR, RT-RPA seems to have lower sensitivity in which its sensitivity could vary from 65% to 94% while its specificity could range from 77% to 100% [124]. Therefore, it is said that RT-RPA could still be less sensitive and specific than qRT-PCR in confirming SARS-CoV-2 infection [124].

Table 5
Comparison of the sensitivity and specificity of RT-RPA in detecting different molecular targets of MERS-CoV and SARS-CoV-2.

Virus type	Molecular targets	Sensitivity (SN)	Specificity (SP)	References
MERS-CoV	N	LOD: 10 RNA molecules (as sensitive as qRT-PCR)	100%	[96]
	N, UpE	UpE: LOD of 12 RNA copies/μL (86%) N: LOD of 1.2 RNA copies/μL (100%)	UpE: 100% N: 100%	[117]
	UpE, ORF 1a	LOD: 3.7^{-1} PFU of MERS-CoV UpE: 98.06% (compared with qRT-PCR) ORF 1a: 99.03% (compared with qRT-PCR)	100%	[118]
SARS-CoV-2	N	LOD: 7.8 RNA molecules/reaction (100% concordance to qRT-PCR)	100%	[120]
	N	LOD: 5 RNA copy/μL sample (98%)	100%	[119]
	N, S	LOD: 0.05 RNA copy/μL sample	Unclear	[97]
	ORF 1ab	96.8% compared to qRT-PCR	Unclear	[121]
	N, RdRp	2.5 RNA copies/μL input For qRT-PCR: LOD was 1 RNA copies/μL input For RT-LAMP: LOD was 10 RNA copies/μL input	100%	[122]
	N, E, ORF 1ab	LOD: 1 RNA copy/μL sample (97%)	100%	[323]
	N, E, RdRp	LOD: 2–15 RNA molecules/reaction (65–94%)	77–100%	[124]

On other hand, a newly developed reverse transcription-insulated isothermal PCR (RT-iiPCR) method targeting the ORF1a and upE genes of MERS-CoV was also developed to assist in the MERS case detection [118]. RT-iiPCR is a fluorescent probe-based nucleic acid detection technique that can be performed in a capillary tube at a single temperature (95 °C) [118]. It was demonstrated that RT-iiPCR assays could detect 3.7^{-1} PFU of MERS-CoV in infected cell culture supernatant and sputum samples, indicating the assay is highly sensitive [118]. The viral nucleic acids of human coronavirus (HCoV)-229E, HCoV-OC43, feline infectious peritonitis virus (FIPV), influenza type A and B virus strains showed no cross-reaction towards the assay and this suggested that RT-iiPCR assay is a highly specific assay that is less likely to produce false-positive test results [118]. RT-iiPCR has also been developed and validated to detect SARS-CoV-2, which has been proven to have comparable sensitivity and specificity to conventional qRT-PCR in confirming SARS-CoV-2 infection [121]. In addition, RT-iiPCR test results are available in less than 1.5 h, while qRT-PCR results might take 3–4 h to be available as it requires additional nucleic acid extraction step [121]. Therefore, RT-iiPCR could be a potential alternative test to confirm the presence of SARS-CoV-2 and MERS-CoV [121].

3.2.1.5. Nucleic acid sequencing. With the advancement in molecular diagnostic technology, nucleic acid sequencing has become one of the options used in diagnosing MERS-CoV and SARS-CoV-2 infections [26,

50,94,125]. Other than qRT-PCR, both WHO and CDC have recommended nucleic acid sequencing as an alternative option used in confirming the presence of MERS-CoV and SARS-CoV-2 (Fig. 2) [29,34,54,57,126]. As such, it is recommended that nucleic acid sequencing can be combined with nucleic acid amplification tests like qRT-PCR in confirming the diagnosis of MERS-CoV and SARS-CoV-2 [29,30,57,126]. For example, to confirm the presence of MERS-CoV, at least two viral genetic targets need to be confirmed using qRT-PCR, but the diagnosis of MERS-CoV infection can be confirmed with only a single positive target detected using qRT-PCR while the second viral genetic target is confirmed using viral nucleic acid sequencing test [29]. For SARS-CoV-2 infection, a nucleic acid sequencing test can also be used to confirm the infection if the nucleic acid amplification test result is questionable or invalid [57].

However, compared to other molecular diagnostic tests like qRT-PCR, nucleic acid sequencing is more costly and time-consuming, and it requires trained personnel to perform and analyze the test findings [17]. In addition, nucleic acid sequencing like whole genome sequencing involves the complete sequencing of the viral genome, where the process is rather complicated and is less suitable for large-scale detection of the virus in a population [17]. Nevertheless, the use of this technique is essential for scientists to identify specific viral gene sequences in order to design suitable primers and probes for subsequent nucleic acid detection tests like qRT-PCR [127].

Besides, nucleic acid sequencing could be used to study the genetic sequences of both MERS-CoV and SARS-CoV-2, which is particularly important in differentiating coronaviruses based on their genetic sequences [5,18,23,128]. On top of that, nucleic acid sequencing is vital to investigate the evolving sequence mutations of the virus which may affect the virus infectivity [17,127,129,130]. A previously reported study has highlighted the unique amino acid substitutions in the ORF 1ab, N, and S proteins would increase MERS-CoV virulence [129]. The study employed whole-genome sequencing to study the viral genetic sequences of eight clinical sample isolates [129]. Similarly, nucleic acid sequencing was also being used to study the specific genetic mutations in SARS-CoV-2, such as D614G mutation, in which this unique nucleotide base substitution increases the transmission rate of the virus [131]. A genome-wide study that investigated over 2400 complete or near-complete genomes of SARS-CoV-2 reported in the GISAID database has found that specific mutations like AA mutations occurred at a higher frequency in the SARS-CoV-2 genomes reported in Europe, followed by Asia then North America [128]. This implied that SARS-CoV-2 that has been circulating worldwide is having different genetic mutations and is highly heterogeneous [128]. Therefore, nucleic acid sequencing could also be used to study the epidemiological distribution and the dynamic of the coronavirus infection [128,132].

In terms of the sensitivity and specificity of nucleic acid sequencing in detecting MERS-CoV, it was reported that the sensitivity of the test could range from 92% to 100%, depending on the occurrence of events like single nucleotide polymorphism (SNP) or nucleotide/sequence mismatch in which these events would reduce the sequencing sensitivity [133]. The specificity of the test in confirming MERS-CoV infection, however, was not further described in the mentioned study [133]. For SARS-CoV-2, a number of protocols and kits were introduced to detect or sequence SARS-CoV-2 (Table 6) [68]. An assay was introduced to sequence the N gene of SARS-CoV and the LOD of the assay was found to be 3000 RNA copies in 1 mL and the test specificity was 100% [134]. For the S gene, two different test flows were established to sequence this genetic region and the targeted genome sequencing could detect 125–250 RNA copies in a reaction with 100% sensitivity and specificity [135,136]. Besides, some assays were introduced to sequence multiple genetic targets of SARS-CoV-2 and in an assay that could detect up to 21 targets of SARS-CoV-2, the LOD of the assay was found to be 2 RNA copies per μL and the assay specificity could range from 90% to 100% [137]. In another protocol that could detect up to multiple targets of SARS-CoV-2, the LOD of the assay was found to be 7–10 RNA copies in 1 mL and the test sensitivity was about 98% [138]. Other than assays that were designed to sequence targeted regions of SARS-CoV-2, some protocols were established to perform whole viral sequencing and the LOD of the assays could range from 3 to 20 RNA copies per assay with a test sensitivity of 90–100% [139–141]. Compared to single target sequencing which was found to have higher sensitivity and specificity (~100%) [135,136], the use of nucleic acid sequencing technology to sequence multiple targets or whole viral genome was reported to have slightly lower sensitivity and specificity (90–100%) [137,138,141]. Some studies have reported that during the genome sequencing process, reads error might occur in one out of a certain number of bases [142,143]. So, the sequencing of multiple targets or sequencing that covers longer genome regions might prone to have more errors that could reduce the test sensitivity and specificity.

Table 6
Comparison of the sensitivity and specificity of nucleic acid sequencing in detecting different molecular targets of MERS-CoV and SARS-CoV-2.

Virus type	Molecular targets	Sensitivity (SN)	Specificity (SP)	References
MERS-CoV	N, UpE, ORF 1ab	92–100% (depending on SNP)	Unclear	[133]
SARS-CoV-2	N	LOD: ~3000 RNA copies/mL	100%	[134]
	S	LOD: 125 RNA copies/mL (100%)	100%	[135]
	S	LOD: 250 RNA copies (100%)	Unclear	[136]
	Whole virus sequence	LOD: 20 RNA copies/sample	Unclear	[139]
	Whole virus sequence	LOD: 3–5 RNA copies/assay	Unclear	[140]
	Whole virus sequence	LOD: 10 RNA copies/assay (>90%)	Unclear	[141]
	N, E, ORF 1a	LOD: 7–10 RNA copies/μL (98%)	100%	[138]
	~21 targets including N, S	LOD: 2 RNA copies/μL (100%)	90–100%	[137]
	Various targets (up to 98 targets)	LOD: 1000 RNA copies/mL	Unclear	[324]

3.2.1.6. CRISPR/Cas-based detection approach. CRISPR/Cas-based technology has recently emerged as one of the potential diagnostic tests to confirm the presence of coronaviruses like MERS-CoV and SARS-CoV-2 [144,145]. The CRISPR/Cas-based assay aids in detecting a specific coronavirus by recognizing its specific genetic sequence and this is followed by the cutting and release of the reporter molecule into the reaction mixture to allow the presence of the virus to be identified [16]. This assay is said to be highly specific and sensitive to detect coronaviruses, and the virus confirmation could be done in as short as 10 min [16,144]. Like the use dPCR to detect MERS-CoV, the use of CRISPR/Cas-based technology in detecting the presence of MERS-CoV is not widely reported, even though some study has incorporated such test to check for the presence of MERS-CoV when the test was originally designed to detect SARS-CoV-2 [144,145]. The use of CRISPR/Cas-based diagnostic techniques was reported to be specific enough to differentiate SARS-CoV-2 from other respiratory pathogens like MERS-CoV [145–149].

To date, multiple studies have reported the use of CRISPR/Cas-based technology to detect SARS-CoV-2 (Table 7) [16,68]. Two studies were previously conducted to use the CRISPR/Cas-based method to detect the N gene of SARS-CoV-2 and it was found that the test could detect up to few RNA copies in a sample with test specificity of 100% [146,150]. When compared the assay finding to that of qRT-PCR, it was shown that the CRISPR/Cas-based detection method results were 95% concordance to the qRT-PCR results [150]. In another study when the

Table 7
Comparison of the sensitivity and specificity of CRISPR/Cas-based tests in detecting different molecular targets of MERS-CoV and SARS-CoV-2.

Virus type	Molecular targets	Sensitivity (SN)	Specificity (SP)	References
MERS-CoV	N, E, S, M, ORF 1ab, RdRp	No data	100% (no cross-reactivity with other pathogens like SARS-CoV-2)	[145–149]
SARS-CoV-2	N	95% concordance to qRT-PCR results	100%	[150]
	N	LOD: few RNA copies/sample	100%	[146]
	S	LOD: 10 RNA copies/reaction (96.23% compared to RT-RPA)	100%	[151]
	RdRp	LOD: 1×10^4 RNA copies/mL	100%	[145]
	N, E	LOD: ~25 RNA copies/μL (93.1%)	98.5%	[152]
	N, E	LOD: ~80 RNA copies/sample	<100% (minor cross-reactivity with other pathogens)	[155]
	N, ORF 1ab	LOD: 6.75 RNA copies/μL	100%	[325]
	N, ORF 1ab	LOD: 1–10 RNA copies/reaction (100% consistent result with qRT-PCR)	100%	[149]
	N, ORF 1ab	LOD: 7.5–25 RNA copies/μL	100%	[326]
	E, ORF 1ab	LOD: 4 RNA copies/μL (100% -ve predictive agreement, 97.14% +ve predictive agreement)	100%	[154]
	N, S, M	LOD: 0.1 RNA copies/μL	100%	[153]
	Unclear	LOD: ~100 RNA copies/mL	100%	[147]

CRISPR/Cas-based method was used to detect the S gene, the LOD of the assay was found to be 10 RNA copies per reaction with 100% specificity and the test results were 96.23% consistent with RT-RPA results [151]. When both N and E regions of the virus were targeted in the CRISPR/Cas-based detection test [152], it was found that the assay sensitivity and specificity were 93.1% and 98.5%, respectively, when compared to the qRT-PCR test results. Combining the findings from the discussed studies, it was demonstrated that the CRISPR/Cas-based detection approach is slightly less sensitive than other methods like qRT-PCR and RT-RPA [146,150–152]. However, a Chinese study reported that the use of a CRISPR/Cas12a-based method that was aimed to target the N and ORF 1ab could detect 1–10 RNA copies per reaction with test sensitivity and specificity of 100% as compared to qRT-PCR [149]. This proved that the CRISPR/Cas-based detection approach could also produce comparable test findings like other SARS-CoV-2 diagnostic tests [149].

In terms of the test specificity, most of the test studies which had employed the CRISPR/Cas-based detection method reported a test specificity of 100%, regardless the test was targeting single or multiple genetic regions of SARS-CoV-2 [145–147,149–151,153,154]. Only some studies had reported that the CRISPR/Cas-based detection assay might be causing some cross-reactivity with other respiratory pathogens and this could be affected by the choice of the molecular targets and cas enzyme [152,155]. For example, a study has reported that the use of LwCas13 to detect ORF 1a, N, and S targets would have a sensitivity of 55% while the use of Cas3a and LbCas12a to detect the N gene would have a specificity of 95% [148]. Nevertheless, the CRISPR/Cas-based detection approach is still new compared to more established methods like qRT-PCR and it is believed that as time passes, more sensitive, reliable, and accurate CRISPR/Cas-based detection assay will be introduced. When compared between different molecular targets, numerous studies had shown that the CRISPR/Cas-based detection assay that was targeting N, S, E, and ORF 1ab could have lower LOD (<100 RNA copies per reaction or mL) [146,149,151,154,155]. Other targets like RdRp, when used in the CRISPR/Cas-based detection assay, however, were found to have a higher LOD which could be as high as 10,000 copies per mL [145]. Thus, in terms of molecular targets, genetic targets like N, E, S, and ORF 1ab should be selected for detection using CRISPR/Cas-based detection assay because these targets could be easier to be detected when the virus load is lower.

3.2.1.7. Other nucleic acid-related tests. In China, a study [156] was conducted to design and evaluate a multiplexed CoVs test coupled with mass spectrometry (MS) sequencing technique that was aimed to target the MERS-CoV N, upE, RdRp, and ORF 1b regions. Such a method was said to be able to detect and differentiate up to 6 known human coronaviruses including MERS-CoV and SARS with LOD of 10–100 RNA copies per reaction and assay specificity of 100% [156]. In this multiplexed coronavirus detection assay, the targeted sequences will be amplified using multiplex PCR, where dideoxynucleotide triphosphates (ddNTPs) are included in the reaction to produce amplification products of various lengths [156]. As the site-specific primers bind to the respective amplicons, it will extend the amplicons by a single base and MassARRAY matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) MS will be used to measure the masses of the extended primers [156].

For SARS-CoV-2, a PCR test that was coupled with MS was also widely used to detect this coronavirus (Table 8) [157–160]. In a Chinese study [159], a PCR test coupled with an MS test was used to detect the N and ORF 1ab genes of SARS-CoV and the detection rate of this assay was noted to be around 75%. On contrary, an American study reported that a similar assay would detect about 1563 RNA copies in mL when it was used to detect N and ORF 1ab genes of SARS-CoV-2 with test sensitivity that ranged from 90% to 100% as compared to qRT-PCR [160]. The drawback of this study was that the tendency of the assay to cross-react with other pathogens was unexplored and thus, the test specificity was unclear [160]. The test specificity of the PCR-MS-based test was also unspecified in another study [158], even though the study reported that such test could detect 400 RNA copies in 1 mL. To further compare the sensitivity and specificity of PCR-MS-based tests in detecting SARS-CoV-2 with other diagnostic methods, a study was conducted in Germany and it was shown that PCR-MS-based assay would produce test

Table 8
Comparison of the sensitivity and specificity of PCR-MS-based tests in detecting different molecular targets of MERS-CoV and SARS-CoV-2.

Virus type	Molecular targets	Sensitivity (SN)	Specificity (SP)	References
MERS-CoV	N, UpE, RdRp, ORF 1b	LOD: 10–100 RNA copies/reaction	100%	[156]
SARS-CoV-2	N, ORF 1ab	Results 100% concordance to qRT-PCR	Specific and no false positive/negative results	[157]
	N, ORF 1ab	LOD: 1562.5 RNA copies/mL (90–100%)	Unclear	[160]
	N, ORF 1ab	LOD: 400 RNA copies/mL	Unclear	[158]
	N, ORF 1ab	Detection rate: 75%	Unclear	[159]
	N, ORF 1ab	LOD: 0.34–110 RNA copies/μL	<100% (cross-reactive with other respiratory pathogens)	[161]

sensitivity and specificity that was comparable and concordance to qRT-PCR test findings [157]. This means the PCR-MS-based assay could have the potential to be further developed as an alternative diagnostic test for coronavirus detection [157]. Since PCR-MS test was shown to provide a promising outcome to detect SARS-CoV-2, a test assay that is dependent on the PCR-MS-based detection approach was approved for use under Emergency Use Authorization (EUA) and this test assay could be used to detect the N and ORF 1ab genes of SARS-CoV-2 [161]. This approved test could detect 0.34–110 RNA copies in 1 μL of sample and its specificity could vary from 90% to 100% because of its potential tendency to cross-react with other respiratory pathogens [161].

On the other side, a paper-based colorimetric DNA assay which is depending on the detection of pyrrolidinyl peptide nucleic acid (acpcPNA)-induced nanoparticle aggregation was being reported as an alternative diagnostic test for MERS-CoV [162]. In the presence of targeted DNA sequences, a DNA-acpcPNA duplex will be formed and this would induce the dispersion of the AgNPs [162]. The nanoparticle dispersion would in turn induce color changes, which could then be observed on the specific paper for further analysis [162]. This paper-based DNA detection approach is a rapid test that could be employed as a Point of Care Testing (POCT) device to directly detect MERS-CoV from the sample itself in an automatic detection manner [162]. This type of assay has been reported to show no cross-reactivity with other CoVs, and its limit of detection for MERS-CoV was found to be 1.53 nM [162]. Thus, this assay could be potentially used as a low-cost diagnostic tool for the rapid screening of infectious diseases [162]. Similarly, the colorimetric assay based on the binding of the nanoparticles with viral nucleic acid was also being developed to detect SARS-CoV-2 [163]. In a study which was conducted in the USA, the scientists developed a type of gold nanoparticle capped with thiol-modified antisense oligonucleotides (ASO), of which the nanoparticle complex is specific to the N gene of the SARS-CoV [163]. When bound to the viral genetic targets, the nanoparticles complexes would form precipitation and the test results could be visualized using the naked eye in less than 10 min [163]. Besides, the assay can differentiate SARS-CoV-2 from other coronaviruses like MERS-CoV, where the detection limit of the test could be as low as 0.18 ng/uL of the RNA isolated from SARS-CoV-2 [163].

3.2.1.8. Rapid, extraction-free nucleic acid tests in detecting SARS-CoV-2. The rapid spreading of SARS-CoV-2 across the world has encouraged many scientists and sci-tech companies to develop and introduce different commercial kits to help in the diagnosis of SARS-CoV-2 [164,165]. Among these commercial diagnostic kits, rapid and extraction-free nucleic acid-based detection assays have been introduced to speed up the diagnostic process without the need to extract RNA from the patient samples (Table 9) [16,68]. These extraction-free nucleic acid tests are currently mainly based on the RT-PCR detection technology, followed by other detection methods like RT-LAMP [68].

For RT-PCR-based extraction-free test kits, currently, the relevant health authorities like FDA, WHO, or CDC have approved at least ten types of such kits and examples of these kits include Xpert®Xpress [166], SalivaDirect [167], SwabExpress [168], PrimeDirect® qRT-PCR Mix [169], Prime® ScriptRT-PCR [169] and few more [16,68]. These test assays or kits could be used to detect various genetic targets of SARS-CoV-2 such as N, E, S, RdRp, and ORF genes and the test sensitivity and specificity would vary greatly from one type of assay to another [166,167,169–172]. In general, the test kits that are made to detect the N gene of the virus are found to have a better sensitivity (>80%) [168,170,173,174] and this is higher compared to test kits that are specifically designed to detect other molecular targets of SARS-CoV-2 like RdRp and E (<70%) [169]. This suggested that the N target could be a better and more reliable target to be used for rapid detection of SARS-CoV-2 as the rapid diagnostic kits are more sensitive to detect this target. In terms of the test specificity, most of the introduced SARS-CoV-2 RT-PCR extraction-free test kits were reported to have high test specificity (>80%) [166–171], except that the test specificity of some kits was unclear like VereRT™ ZeroPrep™ COVID-19 PCR kit [172] and qScript ® XLT qRT-PCR test kit [173]. However, both these test kits have generally low LOD (<12 RNA copies in 1 μL) and high test sensitivity (>85) [172,173].

The increasing use of RT-LAMP in detecting SARS-CoV-2 has also led to the development of an extraction-free test kit that was based on this detection technology and an example of it is the ViroReal ® Kit RT-LAMP SARS-CoV-2 kit [175]. This kit was reported to have test sensitivity and specificity of 95% and 100%, respectively, and the test LOD is 100 RNA copies per reaction [175]. This kit was designed to detect ORF 1ab of SARS-CoV-2 and its superior sensitivity and specificity have made it a good candidate to be employed in rapid diagnosis of SARS-CoV-2 [175]. Next, an Indian company introduced an extraction-free test kit that was based on the CRISPR/Cas-based detection technology and this test assay was aimed to detect S and ORF 1ab of SARS-CoV-2 [176]. This test kit was reported to have a similar LOD as the ViroReal ® Kit RT-LAMP SARS-CoV-2 kit (100 RNA copies in a reaction) and the sensitivity and specificity of this CRISPR/Cas-based test kit was reported to be almost 100% [176]. On the other side, an extraction-free assay that was dependent on the nucleic acid sequencing technology was introduced and approved for use to detect SARS-CoV-2 and it was reported to be able to detect 3200 RNA copies in 1 mL [177]. However, the genetic targets that are targeted by this rapid diagnostic assay and its specificity were unclear [177]. By comparing the test sensitivity and specificity of the extraction-free diagnostic assays that are based on RT-LAMP, CRISPR/Cas, and nucleic acid sequencing [175–177], it is found that these assays have comparable good sensitivity and specificity to detect SARS-CoV-2 by targeting various molecular targets of the virus. Thus, more focus should also be given to extraction-free diagnostic tests that

Table 9
Comparison of the sensitivity and specificity of different rapid, extraction-free molecular test kits/protocols in detecting SARS-CoV.

Name of test kits (manufacturer/country)	Detection technology	Targets	Sensitivity	Specificity	References
Advanta Dx SARS-CoV-2 RT-PCR assay (Fluidigm)	RT-PCR	N	LOD: 6.25 RNA copies/μL	100%	[171]
qScript ® XLT qRT-PCR (Quantabio)	RT-PCR	N	LOD: 6–12 RNA/μL(sensitivity: 85%)	Unclear	[173]
VereRT™ ZeroPrep™ COVID-19 PCR Kit (Veredus)	RT-PCR	N	LOD: 2 RNA copies/reaction	Unclear	[172]
XFree™ COVID-19 qRT-PCR test (BioGX)	RT-PCR	N	>90%	>90%	[170]
FastPlex™ Triplex SARS-CoV-2 Detection Kits	RT-PCR	N	97.9%	95.7%	[174]
SalivaDirect (Yale)	RT-PCR	N	LOD: 1.5 RNA copies/μL	100%	[167]
Xpert ® Xpress (Cepheid)	RT-PCR	N, E	LOD: 0.005–0.02 PFU/mL	100%	[166]
SARS-CoV-2 SANSURE®BIOTECH Novel Coronavirus (Sansure).	RT-PCR	N, ORF 1ab	69.9–94.6%	100%	[169]
SwabExpress (USA)	RT-PCR	S, ORF 1b	LOD: 2–4 RNA/μL (sensitivity: 100%)	99.4%	[168]
PrimeDirect® Probe RT-qPCR Mix (Takara)	RT-PCR	E, RdRp	55.1–91.9%	88%	[169]
PrimeScript®RT-PCR (Takara)	RT-PCR	E, RdRp	69.6–89.2%	100%	[169]
ViroReal® Kit RT-LAMP SARS-CoV-2 (Ingenetix)	RT-LAMP	ORF 1ab	LOD: 100 RNA copies/reaction (95%)	99%	[175]
CASSPIT (India)	CRISPR/Cas	S, ORF 1ab	LOD: 100 RNA copies/reaction (~100%)	100%	[176]
QSanger™-Covid-19 Assay (Swift Biosciences)	Nucleic acid sequencing	Unclear	LOD: 3200 RNA copies/mL	Unclear	[177]

are based on the non-RT-PCR approach to increase the selectability and options for the users to choose and use.

In brief, molecular diagnostic techniques are the main approaches for the detection of both the MERS-CoV and SARS-CoV-2 [16,59,60,66,96,99,105,118,156,157,163]. The advancements in molecular biology and nucleic acids diagnostic technology have enhanced the epidemiological investigations of these viral communicable diseases by enabling the detection of the origins of the coronaviruses, and this also assists in controlling the spread of the infections by isolating the confirmed cases from the community [3,21,30,52,156,178]. In time, it is believed that more sensitive, rapid, easy to perform and cheaper molecular diagnostic kits will be introduced to help detect the presence of human coronaviruses and prevent the spread of the viruses.

3.2.2. *Protein-based tests to detect MERS-CoV and SARS-CoV-2 infections*

Protein-based tests like serological tests and viral antigen detection have been reported as alternative diagnostic tests which could detect both MERS-CoV and SARS-CoV-2 [16,17,183–186,26,32,35,123,179–182]. Generally, compared to nucleic acid-based detection tests, the protein-based test can be less sensitive as it does not involve target amplification [185], and there is a higher possibility of getting false-positive results [183].

Serological tests are applicable to individuals who might have been exposed to MERS-CoV and SARS-CoV-2 or suspected to have these coronaviruses infection since the presence of antibodies to the respective virus reveals that the individual has developed an immune response to the infection [26,186]. Hence, one of the main purposes to conduct serological tests for MERS-CoV and SARS-CoV-2 cases is mainly meant for seroepidemiological study instead of for diagnostic purposes as the presence of virus antigens in infected individuals and the production of antibodies to both coronaviruses could be delayed up to 10 days after the illness onset or the arising of the clinical symptoms [26,55,126,187,188].

A few serological assays were introduced to detect betacoronaviruses, and each of these tests has its pros and cons [16,17,189,190]. For both MERS-CoV and SARS-CoV-2, paired sampling at different time points is recommended for verification purposes, as well as to determine whether the patient is having past or recent acute infection [57,126]. The first sample is to be collected during the illness onset, and the second sample should be ideally taken 21–28 days later, as the increase in the antibody titer could be greater than several-fold in that period [57,126]. For MERS-CoV, a two-phase approach is recommended by both CDC and WHO. First, a rapid screening assay such as immunofluorescence assay (IFA) or enzyme-linked immunosorbent assay (ELISA) is performed to determine the presence of specific antibodies towards viral proteins in the serum samples, followed by a microneutralization (MN) test to confirm the presence of the respective antibodies [54,126]. However for SARS-CoV-2, both CDC and WHO do not recommend the use of virus neutralization test as a routine diagnostic test for Covid-19, as the test requires highly skilled personnel to perform and it takes a longer time for the results to be available [57,185]. Therefore, for SARS-CoV-2, only ELISA is conducted routinely to screen for recent or past infection by this coronavirus as it takes a shorter time [57,185].

3.2.2.1. Diagnosis of MERS-CoV and SARS-CoV-2 using IFA. Anti-MERS-CoV IFA works by illuminating fluorescence of granular structures of viral particles in the cytoplasm of MERS-CoV infected cells, whereas the absence of illumination suggests that the cell is uninfected [191]. This method hinged on the MERS-CoV infected cells as the antigen platform to determine the presence of human anti-MERS-CoV IgG and IgM in the infected individual sera [5,55,192]. The test specificity of IFA was found to be generally low (<85%) and few studies had reported that the use of this assay would likely show cross-reaction with other respiratory pathogens (Table 10) [193,194]. This situation could be improved if the recombinant proteins are used as the antigens in IFA instead of using the whole-virus IFA approach [192]. Although the specificity of IFA is not significant as compared to other molecular diagnostic approaches, however, additional diagnostic information is still available from the unique fluorescent staining patterns in MERS-CoV infected cells as compared to other serological tests like enzyme immunoassay (EIA) [193]. In terms of the test sensitivity of IFA, even though a study has previously reported that IFA was sensitive enough to detect the presence of MERS-CoV in all tested patient samples [193], however, other studies had reported that IFA could have a low detection rate of below 40% [195] and its detection sensitivity would reduce when the samples infected with the virus was further diluted [194]. Another study also reported that when the S protein was employed as the assay target, IFA had a low ability to detect anti-MERS-CoV IgM (30%) and the ability of IFA to detect anti-MERS-CoV IgG was higher (90%). Combining the findings from various studies [192–195], it is said that IFA might not be the best and highly sensitive and specific test to detect the presence of MERS-CoV.

Like MERS-CoV, IFA has also been used in confirming the serological status of the patients infected with SARS-CoV-2 (Table 10) [183,184]. Two studies had reported that the test sensitivity of IFA could be low (<50%) when the assay was used to detect IgG and IgM against SARS-CoV [183,184]. Both these studies used inactivated infected cells for the assay [183,184] and the test specificity of one of the studies was shown to be good (93–100%) in detecting different Ig against

Table 10
Comparison of the sensitivity and specificity of IFA in detecting different targets of MERS-CoV and SARS-CoV-2.

Virus type	Targets/ Sources of samples	Sensitivity (SN)	Specificity (SP)	References
MERS-CoV	S	3/10 (30%) for IgM compared to 9/10 (90%) compared to IgG	Varied due to cross-reactivity with other pathogens in sera of different patients (<100%)	[192];
	Whole virus	100%;	0–83% (depending on cross-reaction with different pathogens)	[193]
	Infected cells	~97.9% at <1:20 dilution	25% of SARS-CoV patients had anti-MERS-CoV antibody reactions	[194]
	Unclear	Detection rate of MERS-CoV = 38% compared to detection rate of MERS-CoV using ELISA = 30% (comparable results	Unclear	[195]
SARS-CoV-2	S	85.7–96.3% (IgG)	High (unclear on the values)	[199]
	Inactivated infected cells	Unclear	~100% (IgG)	[197]
	Inactivated infected cells	76.5–100% (IgG)	86.4% (IgG)	[196]
	Inactivated infected cells	41.9 (IgG), 35.5% (IgM)	Unclear	[183]
	Inactivated infected cells	99% (IgG)	100% (IgG)	[198]
	Inactivated infected cells	41% (IgG)	93% (Ig G), 98.5–100% (IgM/A)	[184]
	Virus from patient sera	91.3% (IgG/A/M) (at Day 14 after onset of symptoms)	98.9% (IgG/A/M) (at Day 14 after onset of symptoms)	[327]

SARS-CoV-2 [184]. In another study where inactivated infected cells were used in the IFA [196], the test sensitivity and specificity ranged from 76% to 100%, and 86.4%, respectively, suggesting that the IFA test results might vary in different studies even though inactivated infected cells were used for the same assay. This hypothesis was further supported when another two studies that used inactivated infected cells showed good sensitivity and specificity in detecting IgG against SARS-CoV-2 (>95%) [197,198]. In a Swiss study that used spike protein-based IFA (recombinant IFA) to detect IgG against SARS-CoV-2, the assay sensitivity was found to be high (>85) [199] and this suggested that the use of either inactivated infected cells or recombinant protein in IFA would produce comparable test findings to detect for the presence of SARS-CoV-2.

3.2.2.2. Diagnosis of MERS-CoV and SARS-CoV-2 using ELISA. Since the start of the MERS-CoV outbreak, an ELISA screening test that used recombinant N and S proteins had been introduced to detect the presence of antibodies specific against the N and S proteins of MERS-CoV [28,200]. Both N and S proteins are highly immunogenic, especially S1 protein is a typical antigen that was used to explore the phylogenetic relationships of coronavirus and epidemiology of MERS-CoV infection [3]. The sensitivity and specificity of ELISA to detect antibodies against MERS-CoV N and S antigens could vary from 20% to 100% and the test findings were depending on the dilution factor of the test samples (Table 11) [200]. Compared to IFA, ELISA was reported to have generally higher sensitivity and specificity in detecting the antibodies against MERS-CoV S protein in which ELISA could detect antibodies against S protein of MERS-CoV in all patient samples while IFA would unable to do so [192]. In another two studies [190,201], ELISA was shown to have superior sensitivity (>90%) to detect IgG against S protein of MERS and the use of this test would not cause false-positive results by cross-reacting with other respiratory pathogens. Therefore, ELISA is said to be a more sensitive and specific test than IFA to evaluate the serological response of the MERS-CoV patients [192].

For SARS-CoV-2, several studies had reported the use of ELISA to detect antibodies against N protein of SARS-CoV and the sensitivity and specificity of such test ranged from 90% to 94%, and, above 97%, respectively (Table 11) [202,203]. Compared to ELISA assay that was aimed to detect antibodies like IgG against the N protein of SARS-CoV-2 [202,203], the use of ELISA test that was focused to detect antibodies against S protein of SARS-CoV-2 showed wide range values of sensitivity and specificity, according to the findings reported from various studies [199,204–207]. Some studies showed that S protein-based ELISA test was less sensitive to detect IgG and IgA against SARS-CoV-2 (<70%) [206,207] while few other studies reported that such test was highly sensitive (at least 90%) to detect IgG, IgA, or IgM against the virus S antigen [199,204,205,208]. Therefore, N protein-based ELISA assay could be a better choice of ELISA test to predict serological response in the SARS-CoV-2 patients as the test results of this assay seem to be more consistent and vary less in different reported studies [202,203] as compared to the S protein-based ELISA test [199,204,206–209]. On the other hand, some studies had reported the use of ELISA test that was aimed to detect different antibodies against different proteins of SARS-CoV-2, and examples of these tests were tests that were focusing to detect antibodies against N and S protein of SARS-CoV-2 [196,204,209–215]. Compared to molecular detection using the qRT-PCR method that showed more consistent test sensitivity and specificity across different reported studies as an example [71,72,87,88], the sensitivity and specificity of ELISA assays in detecting various antibodies (IgG, IgA, and IgM) against different antigen proteins of SARS-CoV-2 demonstrated a high degree of variations in which some studies reported low test sensitivity (<60%) [183,196,216] and specificity (as low as below 80%) [215] whereas some studies would report high test sensitivity and specificity (>85%) [210,211]. Even though the ELISA test might be less sensitive and specific in detecting the presence of SARS-CoV-2 based on the findings from few studies [196,209,215,217], however, this assay was reported to have higher sensitivity and specificity than other serological tests like IFA in detecting the presence of SARS-CoV-2 [183,184].

Table 11
Comparison of the sensitivity and specificity of ELISA in detecting different targets of MERS-CoV and SARS-CoV-2.

Virus type	Targets/ Sources of samples	Sensitivity (SN)	Specificity (SP)	References
MERS-CoV	S	~100%	Varied due to cross-reactivity with other pathogens in sera of different patients (<100%)	[192]
	S	LOD: 0.04–5.9 ng/mL of S protein	Unclear (no significant intra-/ inter-assay variability)	[328]
	S	92.3% (IgG)	100% (IgG)	[201]
	S	~97% at day 21 and above (IgG)	Unclear	[190]
	N, S	varied from ~20–100%;	varied from 0% to 100% (depending on dilution factors)	[200]
SARS-CoV-2	N	LOD: 9.00 ng/μL	High (Unclear on the numerical value)	[329]
	N	94.9% (IgG)	97.1% (IgG)	[202]
	N	90.3% (IgG)	97.9% (IgG)	[203]
	S	88.9–92.9% (IgG)	High (unclear on the values)	[199]
	S	64.1 (IgG), 74.3% (IgA)	95.2 (IgG), IgA (84.2%)	[207]
	S	95% (IgG)	97.8% (IgG)	[205]
	S	65 (IgG/A)	96% (IgG/A)	[206]
	S	95.6–100% (IgG)	86.7–100% (IgG)	[204]
	S (RBD), inactivated virus	53.3–92.1% (IgG)	~99%	[209]
	S (RBD)	92% (IgA), 96% (IgG), 98% (IgM)	99.3%	[208]
	N, S	LOD: 5 pg/μL	100%	[214]
	N, S	88–100% (depending on Ig types)	96.4–100% (depending on Ig types)	[230,330]
	N, S	58.8–96.2% (IgG)	~95% (IgG)	[196]
	N, S	56.3–81.6% (IgG)	99%	[216]
	N, S	74.3–82.2% (IgG), 77.1–80.4% (IgM)	100% (IgG/M)	[213]
	N, S	84.2% (IgG)	100% (IgG)	[212]
	N, S	93.1–98.3% (IgG/total antibody)	86.3–96.4% (IgG/ total antibody)	[211]
	N, S	35.5–61.3% (IgG/M);	Unclear	[183]
	N, S	86.4–87.9% (IgG)	75.7–98.6% (IgG)	[215]
	N, S, total antibody	92.7–99.1% (IgG/total antibody)	98.9–99.9% (IgG/ total antibody)	[210]
	Sera (unclear target)	45% (IgG)	97% (IgG)	[184]
	Sera (unclear target)	69–80.6% (IgA), 48.8–72.9% (IgM), 79.8–83.7% (IgG);	97.6–100% (IgA), 88.1–97.6% (IgM), 97.6–100% (IgG)	[182]

To date, FDA has approved at least ten different types of commercial ELISA test kits under EUA use (Table 12), and generally, these kits could be used for rapid detection of various antibodies against different antigenic targets of SARS-CoV-2 such as N, S, and M proteins [218,219,228,

Table 12
Some of the selected examples of FDA EUA-approved commercial ELISA test kits to detect SARS-CoV-2.

Name of test kits (manufacturer/country)	Antigen targets	Antibody	Sensitivity	Specificity	References
Platelia SARS-CoV-2 Ab Assay (Bio-Rad)	N	Total antibody	92%	99%	[218]
Anti-SARS-CoV-2 ELISA (EUROIMMUN)	S	IgG	90%	100%	[221]
Beijing Wantai SARS-CoV-2 Ab ELISA (Beijing Wantai Co. Ltd)	S	IgG	96.7%	97.5%	[227]
Kantaro Semi-Quantitative SARS-CoV-2 IgG Antibody Kit (Kantaro Biosciences, LLC)	S	IgG	99.1%	99.6%	[223]
Smybiotica COVID-19 Self-Collected Antibody Test System (Symbiotica. Inc)	S	IgG	100%	98%	[225]
COVID-19 ELISA pan-Ig Antibody Test (University of Arizona)	S	Total antibody	97.5%	99.1%	[222]
cPass SARS-CoV-2 Neutralization Antibody Kit (GenScript)	S	Total antibody	100%	100%	[229]
Dimension Vista SARS-CoV-2 Total Antibody Assay (Siemens Healthcare)	S	Total antibody	100%	100%	[219]
Mount Sinai Hospital COVID-19 ELISA Antibody Test (Mount Sinai Hospital)	S	Total antibody	92.5%	100%	[228]
OmniPath COVID-19 Total Antibody ELISA Test (ThermoFisher)	S	Total antibody	96.7%	97.5%	[224]
SARS-CoV-2 Detect IgG/M ELISA (InBios)	N, S	IgG, IgM	96–100%	98–100%	[220]
United Biomedical UBI SARS-CoV-2 ELISA (United Biomedical, Inc)	N, S, M	IgG	89.7%	100%	[226]

220–227]. Examples of these test kits include Anti-SARS-CoV-2 ELISA by EUROIMMUNE [221], Platelia SARS-CoV-2 Ab Assay by Bio-Rad [218], Wantai SARS-CoV-2 Ab ELISA by Beijing Wantai Co. Ltd [227], SARS-CoV-2 Detect IgG/M ELISA by InBios [220] and others. The test sensitivity and specificity of these approved ELISA test kits were noted to be generally good and ranged from 90% to 100%, and above 95%, respectively [218,219,230,220–222,225–229]. Therefore, these test kits would probably help to screen and detect SARS-CoV-2 infection in areas or places where molecular diagnostic tests like qRT-PCR are unavailable [16,230].

3.2.2.3. Diagnosis of MERS-CoV and SARS-CoV-2 using virus micro-neutralization (MN) test. A virus neutralization test is a more definite assay that could be used to detect the presence of neutralizing antibodies specific to MERS-CoV and SARS-CoV-2 as these antibodies would not neutralize the other human coronaviruses [2,57,185,231]. A total of three well-established virus neutralization tests have been published to detect coronaviruses, which are MN assay [35,181,232], pseudoparticle neutralization test (ppNT) [180,233], and plaque reduction neutralization test (PRNT) [123,193,234]. For MN, specific anti-MERS-CoV antibodies were developed and it was used to determine the highest serum dilution required to inactivate the virus cytopathic effect in Vero cells [231]. This method is recognized as a gold standard test to detect specific anti-MERS-CoV as the neutralizing antibodies could be measured [231,235]. However, the MN assay is laborious whereby three to five days of incubation is needed [188,231]. In a study which was aimed to compare the efficiency of MN and ppNT in detecting the neutralizing antibodies against MERS-CoV, it was shown that MN could detect antibodies in 16 out of the 21 samples (76.2%) as compared to ppNT which could detect neutralizing antibodies in 20 out of 21 samples, indicating MN is less sensitive than ppNT in detecting antibodies against MERS-CoV (Table 13) [231]. However, in another study that compared the efficacy between MN, ppNT, PRNT, and ELISA, it was shown that all these four tests were able to detect antibodies in an almost similar number of tested samples at different time intervals, suggesting that the efficiencies of all these four tests were highly correlated and comparable to each other in detecting antibodies against MERS-CoV [190].

Similarly, an MN assay was also being developed to detect SARS-CoV-2 when the virus started to cause a global outbreak (Table 13) [181]. But, as mentioned previously, MN takes at least 3–5 days for the results to be available and it requires highly trained staff to perform the test [181], and thus, both WHO and CDC do not recommend the use of MN in detecting SARS-CoV-2 [57,185]. The ability of MN to detect neutralizing antibodies against SARS-CoV-2 was reported to be highly dependent on the dilution factor used in a test [236,237]. Some studies reported that MN could only detect antibodies against SARS-CoV-2 at a dilution factor of 1:20 and it would not be able to detect the antibodies when the testing reaction was diluted further [237]. In another study, it was reported that the LOD of MN was at a dilution factor of 1:40 [236], implying that the LOD of this assay might vary across different assay settings. Compared to other protein-based tests like ELISA, it was shown that MN could serve as an alternative test to confirm the presence of antibodies against SARS-CoV-2 when ELISA was unable to do so, suggesting MN has superior sensitivity than ELISA [238]. Thus, in the development and validation of the sensitivity and efficiency of various immunoassays or ELISA to detect antibodies against SARS-CoV-2, MN was always used as a standard test to be compared with [236,239,240].

3.2.2.4. Diagnosis of MERS-CoV and SARS-CoV-2 using pseudoparticle neutralization test (ppNT). The main advantage of ppNT in MERS-CoV and SARS-CoV-2 diagnosis is that biosafety level (BSL)-3 containment is not compulsory, as no actual Risk Group 3 virus is being used [188, 190]. ppNT which utilizes HIV/MERS spike pseudoparticles to infect the Vero E6 cells was reported to have a shorter incubation time than MN assay [231]. For the time being, ppNT assay is mainly applied in the seroepidemiology study which involves animals and livestock, where the assay demonstrates a good correlation with the gold standard test, MN [2186,231]. Besides, ppNT assay was demonstrated to produce a reliable testing outcome when it was used in human samples during the first six weeks of MERS-CoV infection [190]. Compared to other

Table 13
Comparison of the sensitivity and specificity of micro-neutralization (MN) test in detecting different targets of MERS-CoV and SARS-CoV-2.

Virus type	Targets/Sources of samples	Sensitivity (SN)	Specificity (SP)	References
MERS-CoV	Whole virus	16/21 (76.2%) compared to 20/21 (95.2%) of ppNT;	100%	[231]
	Whole virus	97% at day 21 and above (IgG); comparable efficiency with ppNT, PRNT and ELISA	Unclear	[190]
SARS-CoV-2	Whole virus	Sensitivity of three immunoassays to MN = 63.1–91.1%	Specificity of six immunoassays to MN = 100%	[239]
	Whole virus	Varied and weaker compared to ELISA (most tests only detected virus at 1:10/1:20 dilution)	Unclear	[237]
	Whole virus	Able to confirm presence of SARS-CoV-2 when ELISA was unable	Unclear	[238]
	Whole virus	LOD for MN: 1/40 dilution Sensitivity of six immunoassays to MN = 43.8–87.8%	Specificity of six immunoassays to MN = 68.3–97.5%	[236]
	Whole virus	≥50% compared to ELISA	Unclear	[331]
	Whole virus	Sensitivity of six immunoassays to MN = 69–100%	Unclear	[240]

protein-based tests like MN and PRNT, ppNT was said to produce comparable test findings with these protein tests [190]. Therefore, when protein tests like ELISA and MN were unable to detect any antibodies against MERS-CoV, ppNT could be used as another optional test to confirm the presence of MERS-CoV [241,242]. In terms of its LOD, a study has reported that ppNT was able to detect antibodies against MERS-CoV in more than 90% of the test samples when the dilution factor was set at 1:40 [233]. Compared to another study that has reported that MN could detect anti-SARS-CoV-2 at a dilution factor of 1:40 [236], ppNT was said to have a similar detection limit with that of MN when used to detect another coronavirus type. This further supported that ppNT has a sensitivity that is not inferior to MN (Table 14). For SARS-CoV-2, a ppNT test that could be conducted in the BSL-2 facility was reported and the limit of detection was found to be 43 and 22 for mouse and human samples, when the cutoff values were set at 50 and 30, for mouse and human samples, respectively [180]. Besides, the ppNT assay was also shown to be highly reproducible with low intra- and inter-assay variations of 15.9% and 16.2%, respectively [180]. In the clinical setting, ppNT could be used to measure the neutralizing antibodies against SARS-CoV and this is important to evaluate the remaining antibody levels in the patient body post-infection [243,244]. The findings from two ppNT-based studies (Table 14) reported that the neutralizing antibodies against the pseudoparticle virus would start to drop within 4–8 weeks post-infection [243,244], suggesting that the patients might have a risk of being re-infected. In short, the introduction of ppNT has helped to reduce the risk of viral infection among the testing personnel as it does not require the handling of live, infectious viruses. [180,188,190]. Its good accuracy and versatility might ease the development of a viral vaccine or drug [180].

Table 14
Comparison of the sensitivity and specificity of pseudoparticle neutralization test (ppNT) in detecting different targets of MERS-CoV and SARS-CoV-2.

Virus type	Targets/ Sources of samples	Sensitivity (SN)	Specificity (SP)	References
MERS-CoV	S protein pseudoparticle	MERS-CoV undetected by MN could be detected by ppNT (% sensitivity unclear)	Unclear	[241]
	Virus pseudoparticle	118/131 (90%) (at 1:40 dilution)	100%	[233]
	Virus pseudoparticle	94% at day 21 and above (IgG)	100%	[190]
	Unclear	ppNT was used to confirm MERS-CoV infection when ELISA showed false +ve/-ve findings	Unclear	[242]
SARS-CoV-2	Virus pseudoparticle	Neutralizing antibody levels would drop after 8 weeks of infection but still measurable up to 8 months. Pseudovirion improved infectivity of SARS-CoV-2 neutralization assay	Unclear	[244]
	Virus pseudoparticle	LOD: 1:100 dilution; LOD of 22.1 and 43.2 for human and mice sera when 120 negative samples were used	Low intra- and inter-assay variations of 15.9% and 16.2%, respectively	[180]
	Virus pseudoparticle	Unclear but 1/3 of the patients would lose the antibodies against pseudoparticle a month after onset of symptoms	Unclear	[243]

3.2.2.5. Diagnosis of MERS-CoV and SARS-CoV-2 using plaque reduction neutralization test (PRNT). PRNT is considered as another gold standard test to determine the neutralizing antibody titers against an infectious agent [123]. For MERS-CoV, PRNT exhibits slightly higher sensitivity in detecting the early antibody responses in MERS-CoV infection compared to MN and ppNT neutralization assays [190]. Hence, it is recommended that PRNT be conducted in patients with poor serological responses as it gives great sensitivity to other neutralization assays [190]. In another study [192], PRNT was reported to have comparable sensitivity with other protein-based tests such as IFA and ELISA in detecting the antibodies against MERS-CoV. However, in terms of its specificity, even though the authors suggested that PRNT could be the most specific test to detect MERS-CoV than other tests, but cross-reactivity with other pathogens cannot be ruled out [192]. Nevertheless, the same study has proven that the antibodies against MERS-CoV would fade over time in the human body post-infection and this suggested that the patient has a risk of re-infection with MERS-CoV in the future [192]. For SARS-CoV-2, several studies had been conducted to compare the sensitivity and efficacy of PRNT to detect antibodies against SARS-CoV-2 (Table 15), and in one of the studies [217], it was shown that PRNT has comparable sensitivity with ELISA in detecting antibodies against SARS-CoV-2. In another study that compared the efficiency and sensitivity of three tests, namely, ELISA, MN, and PRNT, the authors reported that PRNT is more sensitive than MN and ELISA in detecting anti-SARS-CoV-2 [245]. The superiority of PRNT in detecting antibodies against SARS-CoV-2 was

Table 15
Comparison of the sensitivity and specificity of plaque reduction neutralization test (PRNT) in detecting different targets of MERS-CoV and SARS-CoV-2.

Virus Type	Targets/ Sources of samples	Sensitivity (SN)	Specificity (SP)	References
MERS-CoV	Whole virus	Comparable sensitivity with IFA and ELISA	Varied due to potential cross-reactivity with other pathogens in sera of different patients (<100%)	[192]
	Whole virus	97–100% at day 21 and above (IgG)	Unclear	[190]
SARS-CoV-2	Whole virus	76.5–100%	Unclear	[196]
	Whole virus	Over 99% of the sera remained seropositive for both 90% ($PRNT_{90}$) and 50% ($PRNT_{50}$) neutralization endpoints after 61 days of infection	Unclear	[123]
	Whole virus	Neutralizing activity was 93% for $PRNT_{50}$ and 54% for $PRNT_{90}$ at around day 33 after onset of symptoms	Unclear	[332]
	Whole virus	100%	Unclear	[206]
	Whole virus	100% compared to ELISA	Unclear	[217]
	Whole virus	PRNT > MN and ELISA (% of sensitivity unclear)	Unclear	[245]
	Whole virus	Virus neutralization test vs PRNT (89.8–99.0%)	Virus neutralization test vs PRNT (100%)	[246]

further shown when a Canadian study employed PRNT as a reference test to evaluate the sensitivity and specificity of the virus neutralization test [246]. Compared to PRNT, the virus neutralization test was demonstrated to have a slightly lower sensitivity of 89.8–99% whereas in terms of the test specificity, both neutralization test and PRNT were found to have similar and comparable specificity (~100%) [246]. In clinical use, like in the case of MERS-CoV, PRNT was also used to evaluate the levels of neutralizing antibodies in the bodies of the patients infected with SARS-CoV-2 and it was shown that patients who had more severe infections would have higher peak levels of $PRNT_{90}$ and $PRNT_{50}$ after months of infection, compared to patients with mild infection or asymptomatic patients [123]. This suggested that the levels of neutralizing antibodies were correlated to disease severity and SARS-CoV-2 is possible to create robust neutralizing antibodies in patients with severe infection [123]. A limitation that was not well-explored in the study [123] is that the authors did not specify whether other neutralization tests would still be able to detect the neutralizing antibody in the patients after two months of infection, and thus, it is inconclusive on whether PRNT is more superior and sensitive than other neutralizing tests in detecting the antibody responses towards SARS-CoV-2 after months of infection.

3.2.2.6. Viral antigen detection for MERS-CoV and SARS-CoV-2. A viral antigen test aims to detect the specific antigen which is present on the virus surface. This type of assay could also be used to detect both MERS-CoV and SARS-CoV-2 [179,247]. In a study that used 129 nasopharyngeal aspirates which were positive to various human respiratory viruses, it was found that the use of MERS-CoV-nucleocapsid protein (NP)-specific monoclonal antibodies were sensitive and specific in detecting MERS-CoV antigen, in which the test specificity was almost 100% [179]. Besides, the MERS-CoV-NP-specific monoclonal antibodies could also detect the presence of live MERS-CoV in simulated nasopharyngeal aspirates samples after rounds of serial dilutions, when the tissue culture infectious dose ($TCID_{50}$) per 0.5 mL was at least 10 [179]. However, the sensitivity and validity of the virus antigen test reported in this study were not compared with other molecular tests like qRT-PCR [179]. In the year 2016, a similar group of researchers compared the sensitivity of viral antigen test to detect spike protein of MERS-CoV with the test sensitivity of ELISA to detect antibodies against MERS-CoV [100]. The report showed that the test sensitivity of antigen test was about 81% as compared to ELISA and it was estimated to be at least 25–100-folds less sensitive than ELISA to detect for the presence of MERS-CoV (Table 16) [100]. However, the viral antigen test was proven to have high specificity to detect MERS-CoV and would produce results in less than half an hour [100].

On the other hand, a viral antigen test was also being used to detect the presence of SARS-CoV-2 (Table 16) [68,248]. Like MERS-CoV, virus antigen test was generally reported to have lower sensitivity (<80%) in detecting the specific antigen of SARS-CoV-2, especially when this test was compared to the molecular diagnostic test like qRT-PCR [249–252] In terms of the test specificity, antigen test was found to be most specific (>90%) to detect SARS-CoV-2 without creating cross-reaction when other respiratory pathogens were present [249–257]. However, there was also some study which had reported that the antigen test results could have low specificity (as low as 46.2%) and this suggested that antigen test could be used in rapid screening of the SARS-CoV-2 infection but the use of other confirmatory tests like qRT-PCR should be conducted to confirm the viral infection [258]. As for the molecular target that is frequently used in the virus antigen test, both N and S antigens of SARS-CoV-2 are the commonly employed antigenic targets in the virus antigen test, and the LOD to detect these antigenic targets could range from 100,000 RNA copies in 1 mL to 2.86 × 107 RNA copies in a single swab [253,256]. Therefore, compared to molecular tests like qRT-PCR which could detect as low as few RNA copies in 1 μL [68,78], it is said that more viral copies are needed to be present in the test samples for the virus to be detected by the viral antigen test [253,256].

Table 16
Comparison of the sensitivity and specificity of virus antigen test in detecting different targets of MERS-CoV and SARS-CoV-2.

Virus type	Targets/ Sources of samples	Sensitivity (SN)	Specificity (SP)	References
MERS-CoV	N	+ve results for samples with 10 $TCID_{50}$/ 100 μL;	100%	[179]
	S	$10^{3.7}$–$10^{4.2}$ $TCID_{50}$/mL (81%, ~100 times less sensitive than ELISA)	100%	[100]
SARS-CoV-2	N	98.33%	98.73%	[257]
	N	LOD95: $2{\cdot}07 \times 10^6$ and $2{\cdot}86 \times 10^7$ copies/ swab	98.5–100%	[256]
	N	45.4–50.3%	97.7–97.8%	[250]
	N, S	LOD: 0.31–102 $TCID_{50}$/mL (84–97.7%) (depending on antigen type)	93.9–100%	[230,248]
	N, E, S, M	LOD: 100,000 RNA copies/mL (78.8%)	99.7%	[253]
	Unclear	96.52%	99.68%	[255]
	Unclear	11.1–45.7% (qRT-PCR>virus culture>antigen test)	~100% (no cross-reactivity with other pathogens)	[249]
	Unclear	81%	99.1%	[254]
	Unclear	69.86%	99.61%	[251]
	Unclear	72.5%	99.4%	[252]
	Unclear	88.9–100%	46.2–100%	[258]

Rapid case detection is essential to identify subjects who have been infected with SARS-CoV-2 and this ensures the infected people will be isolated from other people to prevent the virus from spreading in a community [57,58]. For rapid virus screening, multiple rapid viral antigen tests have been approved by FDA for EUA use (Table 17) and examples of these tests include BinaxNOW COVID-19 Ag Card Home Test by Abbott Diagnostic Scarborough Inc [259], CareStart COVID-19 Antigen Test by Access Bio Inc [260], GenBody COVID-19 Antigen Test by GenBody Inc [261], InteliSwab COVID-19 RAPID Test Pro by OraSure Technologies Inc [262]. In general, these test kits were developed to detect N and S antigens of SARS-CoV-2 and the test sensitivity and specificity are ranging from 80% to 100%, and 90–100%, respectively [259–261,263–268]. In terms of rapid virus screening and detection, the sensitivity and specificity of these tests are considered to be good to enable the local medical personnel to screen for any suspected SARS-CoV-2 infection in a community [16,17,230]. For any indeterminate test finding, further test like qRT-PCR is recommended to confirm whether a subject has contracted the virus [57,247].

To sum up, for this section, both ELISA and virus antigen tests can be done rapidly and both assays could be completed in a shorter time (<1 h) as compared to viral neutralization tests [17]. However, the virus antigen test is said to have lower accuracy, sensitivity, and is less reliable when compared to ELISA [17,68]. In terms of invasive-ness, even though the use of nasopharyngeal swabs or aspirates for virus antigen detection sounds more user-friendly, but the nasopharyngeal samples collection process could be sometimes invasive and could lead to bleeding in the upper nasopharyngeal regions [269]. Thus, a different new method like the saliva specimen collection approach [269] has been introduced to screen for the presence of the virus antigen in the patient specimens. But, this new method was shown to have poorer sensitivity in detecting the coronaviruses, for example, when saliva specimen was used in the antigen testing for SARS-CoV-2, the sensitivity of the test was just about 21% as compared to the nasopharyngeal swab antigen test which could show a sensitivity of 52% [269].

Table 17
Some of the selected examples of FDA EUA-approved commercial rapid antigen test kits to detect SARS-CoV-2.

Name of test kits (manufacturer/country)	Antigen targets	Sensitivity	Specificity	References
BD Veritor System for Rapid Detection of SARS-CoV-2 (Becton, Dickinson and Company)	N	84%	100%	[265]
BinaxNOW COVID-19 Ag Card Home Test (Abbott Diagnostics Scarborough Inc)	N	97.1%	98.5%	[259]
CareStart COVID-19 Antigen Test (Access Bio, Inc)	N	88%	100%	[260]
Ellume COVID-19 Home Test (Ellume Limited)	N	95%	97%	[267]
GenBody COVID-19 Antigen Test (GenBody Inc)	N	90%	98%	[261]
InteliSwab COVID-19 RAPID Test Pro (OraSure Technologies, Inc.)	N	85%	99%	[262]
LumiraDx SARS-CoV-2 Ag Test (LumiraDx UK Ltd)	N	97.6%	97%	[268]
Omnia SARS-CoV-2 Antigen Test (Qorvo Biotechnologies, LLC.)	N	89.5%	100%	[333]
SCoV-2 Ag Detect™ Rapid Test (InBios International Lnc)	N	86.67%	100%	[264]
Sienna-Clarity COVID-19 Antigen Rapid Test Cassette (Salofa Oy)	N	87.5%	98.9%	[263]
Status COVID-19/Flu Test (Princeton BioMeditech)	N	93.9%	94%	[266]
COVID-19 Antigen MIA (Celltrion USA)	S	94.4%	100%	[334]

3.2.3. Virus culture of MERS-CoV and SARS-CoV-2

The concept of virus-cell culture involves the use of cell lines to culture virus isolated from patients who are suspected to have MERS-CoV and SARS-CoV-2 infections [32,35]. Examples of commonly used cell lines for virus-cell culture include Vero E6 and LLC-MK2 cells, in which the former is derived from the kidney of African green monkey while the latter is derived from the kidney of rhesus monkey [16,270]. Following virus replication, the infected cells might die secondary to cellular apoptosis and the cells which survive after virus replication would show cellular morphology similar to healthy, uninfected cells, and the survived cells would continue to support the virus production [16]. The entry of both MERS-CoV and SARS-CoV-2 into host cells is mediated and enhanced by the proteolytic cleavage of the viral S protein by a host protein called type II transmembrane serine protease (TMPRSS2) [271,272]. A group of Japanese researchers has engineered a Vero E2 cell line which expresses TMPRSS2, where the use of such cell lines would enhance the viral RNA production by at least ten-folds for both MERS-CoV and SARS-CoV-2 [271,272]. Hence, such cell lines can be potentially used in diagnostic laboratories to speed up the virus identification process.

Apart from being used in virus identification, virus-cell culture has also been widely used in the anti-viral drug development, in which anti-viral agent will be administered to the cultured cells to determine whether it would help to inhibit virus replication [273,274]. As such, virus-cell culture has been extensively employed to test the potential anti-viral agents against SARS-CoV-2 [273] and influenza virus [274]. The limitation of virus-cell culture is that the identification of virus using this method requires further tests like qRT-PCR or nucleotide sequencing to confirm the virus identity which is isolated from the clinical samples [275]. Besides, this method is time-consuming and insensitive, and it requires trained and specialized personnel to perform the procedure [35, 276].

3.3. Radiological diagnosis of MERS-CoV and SARS-CoV-2 infections

Chest radiography (CXR) and computed tomography (CT) scans are the commonly used radiological investigations that could be used to help identify chest infection caused by both MERS-CoV and SARS-CoV-2 [277–279]. For pneumonia caused by MERS-CoV, it was reported that both CXR and chest CT would show features of organizing pneumonia evidenced by the presence of multifocal ground glass opacities [278, 279]. Similarly, the CXR and chest CT in the patients infected with SARS-CoV-2 would also show features of ground-glass opacities [17, 277], suggesting chest infection caused by coronavirus infection is more commonly presented with ground-glass appearances under radiological imaging. A comparative review has reported that MERS-CoV infection is more prone to cause unilateral chest infection and pleural effusion, while SARS-CoV-2 infection is more likely to cause bilateral chest infection [277]. Therefore, the laterality involvement and the presence of pleural effusion could be potentially used to differentiate MERS-CoV and SARS-CoV-2 chest infection [277]. Another common radiological feature that could be found in the chest imaging of the patients infected with MERS-CoV and SARS-CoV-2 is the presence of signs of pulmonary fibrosis, where this predominant finding is obvious for patients who have recovered from coronavirus infection [277].

Even though chest infection caused by both MERS-CoV and SARS-CoV-2 would produce some typical features under radiological imaging, however, these radiological features are non-exclusive to the respective viruses. For example, pleural effusion has been being reported in 153 out of 473 patients (32.3%) infected with SARS-CoV-2 in China [280]. Besides, pneumonia with ground-glass opacity could also be caused by other viruses like cytomegalovirus [281], and pleural effusion could be present in pneumonia caused by other causative agents such as SARS-CoV [277] and bacteria like *Pseudomonas aeruginosa* [282]. Likewise, pulmonary fibrosis could also be caused by other infectious agents such as herpes and Epstein-Barr viruses [283]. In short, the presence of ground-glass opacity, pleural effusion, and pulmonary fibrosis under radiological imaging could not be used to confirm coronavirus chest infection.

4. Detections of different variants of SARS-CoV-2

The COVID-19 pandemic has hit mankind for more than 16 months since its first appearance at end of 2019 [284–287]. During this period, the SARS-CoV-2 virus was found to undergo continuous mutations in their genetic sequences and different variants of SARS-CoV-2 have spread to many different countries [284–289]. The appearance of these different SARS-CoV-2 variants has led to the increased virus transmissibility and reduced effectiveness of the currently available vaccines in curbing the spreading of SARS-CoV-2 [284–287,290–295]. To ease the classification and grouping of the different virus variants, the relevant global and local health authorities have decided to divide the SARS-CoV-2 variants into four main groups (Table 18), namely, variant of high consequence (VOHC), variant of concern (VOC), variant of interest (VOI) and variant under monitoring (VUM) [284,287,296].

VOHC is defined as a variant that would significantly reduce the effectiveness of the preventive measures and medical countermeasures that are designed to eradicate and prevent the transmission of the concerned virus [284,287,297]. To date, there is no any SARS-CoV-2 variant that is classified as VOHC [284,287]. On the other hand, several SARS-CoV-2 variants have been grouped under (VOC) and these include alpha (B1.1.7), beta (B.1.351), delta (B.1.617.2), and gamma (P.1) [284, 287]. VOC is said to have increased transmissibility and the specific mutations which they have acquired would reduce the effectiveness of the currently available vaccines, and there is much scientific evidence to suggest that these variants would increase the disease severity [287–289,297–299]. The spike protein mutations which the VOC could

Table 18
Variant of concern (VOC) and variant of interest (VOI) of SARS-CoV-2.

	Variant of concern (VOC)				Variant of interest (VOI)				
Variant type	B.1.1.7 (Alpha)	B.1.351 (Beta)	B.1.617.2 (Delta)	P.1 (Gamma)	B.1.427 and B.1.429 (Epsilon)	B.1.525 (Eta)	B.1.526 (Iota)	B.1.617.1 (Kappa)	**P.2 (Zeta)**
Origin	United Kingdom	South Africa	India	Brazil and Japan	California, United States of America	United Kingdom and Nigeria	New York, United States of America	India	Brazil
Protein changes/ mutations	69del, 70del, 144del, (E484K[b]), (S494P[b]), N501Y, A570D, D614G, P681H, T716I, S982A, D1118H (K1191N*)	D80A, D215G, 241del, 242del, 243del, K417N, E484K, N501Y, D614G, A701V	T19R, (G142D[a]), 156del, 157del, R158G, L452R, T478K, D614G, P681R, D950N	L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I	L452R, D614G, S13I, W152C	A67V, 69del, 70del, 144del, E484K, D614G, Q677H, F888L	L5F, (D80G[a]), T95I, (Y144[-a]), (F157S[a]), D253G, (L452R[a]), (S477N[a]), E484K, D614G, A701V, (T859N[a]), (D950H[a]), (Q957R[a])	(T95I), G142D, E154K, L452R, E484Q, D614G, P681R, Q1071H	E484K, (F565L[a]), D614G, V1176F
Effects on transmissibility	Increased by 50%	Increased by 50%	Increase (% unclear)	Unclear	Increased by 20%	Unclear	Unclear	Unclear	Unclear
Reduce neutralization by sera/ antibody	Low impact	Significant reduced neutralization	Potential reduced neutralization	Significant reduced neutralization	Moderate reduced neutralization	Potential reduced neutralization	Reduced neutralization	Potential reduced neutralization	Potential reduced neutralization
References	[284,285,294–300,335–337,286,338–343,287–293]								

[a] Amino acid changes were detected in some of the tested sequences but not all of the tested samples. Till date, there is no any variant that is classified as the variant of high consequence (VOHC). The variant list is not limited to the above variants and the list can be expanding from time to time.

have acquired include E484K, D614G, N501Y, L452R, and many more, and these mutations could increase the virus ability to bind to the human cell receptor to increase their ability to enter and infect the human cells [284,287,297] VOI is a variant that is considered to be less virulent than VOC and they have the potential to cause increased transmission of the virus, worsened the disease severity and could probably reduce the virus neutralization by antibodies produced from past infection or vaccination [284,287]. Examples of VOI include epsilon (B.1.427 and B.1.429), eta (B.1.525), iota (B.1.526), kappa (B.1.617.1), and zeta (P.2) [284,287]. On contrary, VUM is a variant that was detected through some variant screening and they could have properties similar to VOC but there was limited scientific evidence to group them under VOC [287]. Currently, several variants are classified as VUM and these include lambda (B.1.351+E516Q or B.1.1.7+L452R or B.1.1.7+S494P or C.36+L452R or AT.1 or C.37), Iota (B.1.526.1 or B.1.526.2), and Zeta (B.1.1.519 or AV.1 or P.1+P681H or B.1.671.2+K417N) [287].

Detecting and understanding the variant type of SARS-CoV-2 is essential to help to contain the transmission of the virus in a specific region because certain variants like alpha and delta virus strains are known to accelerate the virus infection in a community and it is well-established that there is reduced in the effectiveness of the currently available vaccines to neutralize these viruses [289–292,297,298,300]. Currently, several types of detection tests could be performed to detect or identify different SARS-CoV-2 variants (Table 19) [296]. One of them is RT-PCR in which a target failure or weak target detection could suggest the presence of a mutation like deletion [296,301]. This technique, however, has a limitation because it cannot differentiate different VOC or VOI because mutations like N501Y are almost present in all variant types [296]. In terms of the test sensitivity, it was reported that the qRT-PCR method could detect virus variants at as low as 4–10 RNA copies per reaction [302,303], while some studies reported that the LOD of RT-PCR could be ranging from 100 to 1000 RNA copies in 1 mL [301, 304]. By comparing to other detection methods like nucleic acid sequencing, qRT-PCR was shown to have comparable sensitivity and specificity in detecting the SARS-CoV-2 variant types [301,305].

Nucleic acid sequencing remains an essential gold-standard method to detect different SARS-CoV-2 variants because this technique allows the detailed sequencing of the viral genome or specific gene to unravel any mutational changes [296]. Currently, few studies have reported the use of nucleic acid sequencing to identify and differentiate various VOC and VOI [296,306,307], and its LOD was reported to be around 19 RNA copies per reaction [308]. Compared to qRT-PCR, nucleic acid sequencing is said to be a more objective and unbiased test to detect the SARS-CoV-2 variant because the test user does not know about the virus information before the test [296]. Besides, some studies reported the use of qRT-PCR to screen for the presence of a specific target mutation, followed by nucleic acid sequencing tests like Sanger sequencing to confirm the mutation [309,310]. This approach could help to speed up test time and reduce test cost as there is no need to run whole-genome sequencing of the virus to confirm its identity [310]. On contrary, SNP genotyping was also reported to be employed in detecting different SARS-CoV-2 variants and this assay was found to have the ability (>62%) to distinguish two virus variants of different genotypes [311].

Other than RT-PCR and nucleic acid sequencing, the RT-LAMP-based approach was also being reported to be used in the detection of different SARS-CoV-2 variants [312,313]. This assay was used to identify various variants like Alpha (B.1.1.7) and Gamma (P.1) by detecting mutations like 69del, 70del, and N501Y [312,313]. The LOD of this test approach was reported to be ranging from 39 to 10,000 RNA copies in a reaction [313], and the test sensitivity and specificity were shown to be around 97%, and 100%, respectively [312]. On the other side, a molecular diagnostic approach that is based on the CRISPR/Cas-based detection technique was also being employed in identifying different SARS-CoV-2 variants and the LOD of this test was reported to vary from 10 to 1000 RNA copies in a reaction [155,314,315]. The specific binding and interaction between the Cas enzyme and the RNA strand allow the

Table 19
Detection of various SARS-CoV-2 variants using different methods.

Methods	Types of detectable variants	Examples of detectable mutations	Key findings	References
qPCR/PCR	B.1.1.7 (Alpha)	N501Y	LOD: 4 RNA copies/reaction, Sensitivity and specificity: 100% (MC)	[303]
	B.1.1.7 (Alpha), B.1.351 (Beta),	N501Y, E484K	LOD: 10 RNA copies/reaction	[302]
	B.1.1.7 (Alpha), B.1.351 (Beta), P.1 (Gamma)	N501Y	LOD:5000 RNA copies/mL, sensitivity and specificity = 100%	[301]
	B.1.1.7 (Alpha), B.1.351 (Beta), P.1 (Gamma)	K417N, N501Y	Sensitivity of PCR to detect VOC could be 10% better than whole genome sequencing	[305]
	Almost all variants	N501Y, E484K, K417N	RT-PCR and melt curve analyses were able to detect 80% of alpha, 5% of beta, 2% of gamma and 13% of non-variants of SARS-CoV-2 from 989 samples	[344]
	Almost all variants	N501Y, D614G	LOD: 100 RNA copies/mL, specificity unclear	[304]
	Almost all variants	N501Y, 69del, 70del, K417N, and E484K	Sensitivity and specificity unclear	[345]
	B.1.1.7 (Alpha), B.1.351 (Beta), P.1 (Gamma)	E484K	LOD: 1–100 copies/μL Sensitivity and specificity: cannot differentiate all VOC/VOI	[69]
Nucleic acid sequencing	B.1.1.7 (Alpha)	N501Y	LOD:18.5 RNA copies/reaction, sensitivity and specificity unclear	[308]
	B.1.1.7 (Alpha)	P681H, E484K	Nucleic acid sequencing is able to differentiate B.1.1.7 (58%) and non-B1.1.7 infection (42%)	[306]
	B.1.1.7 (Alpha), B.1.1.162. B.1.1.267, B.1.1	D614G, A222V, S982A, T716I, P681H, A570D, E583D	Nucleic acid sequencing was able to detect more than 60% of the SARS-CoV-2 variants from different samples collected from different infection waves.	[307]
	B.1.351 (Beta)	K417N, E484K, N501Y	Number of accumulated amino acid changes in whole genome = 341	[338]
PCR + Nucleic acid sequencing	B.1.1.7 (Alpha)	N501Y, S477N and D614G	LOD: Only samples with Cq value < 25 for N gene could be detected using WGS. Specificity: Unclear	[309]
	Almost all variants	69del, 70del, Y144del, N501Y and A570D	Compared to WGS, RT-PCR and sanger sequencing require shorter time and lower cost. LOD unclear	[310]
SNP genotyping	Almost all variants	Unreported	>62% to be able to distinguish two variants of different genotypes	[311]
RT-LAMP	P.1 (Gamma), P.2 (Zeta)	N501Y, E484K/Q, K417N/T	Sensitivity: 97%, Specificity: 100% (based on N/E targets)	[312]
	B.1.1.7 (Alpha)	69del, 70del	LOD: 39–10,000 RNA copies/reaction	[313]
CRISRP/Cas-based technique	B.1.1.7 (Alpha), B.1.351 (Beta), P.1 (Gamma)	N501Y	LOD, sensitivity and specificity unclear	[346]
	Almost all variants have D614G	D614G	LOD: 10 RNA copies/reaction	[314]
	Other variants	E174R/S542R/K548R, S254F	LOD: 50–1000 RNA copies/reaction, specificity: 100%	[315]
	Almost all variants have D614G	D614G	LOD: 82 RNA copies/reaction, % specificity unclear	[155]
Antigen test	B.1.1.7 (Alpha), B.1.351 (Beta),	Unclear	LOD: $1.7 \times 10^5 - 6.6 \times 10^7$ RNA copies/mL Sensitivity and specificity: Unclear	[316]
Antigen and antibody test	B.1.1.7 (Alpha), B.1.351 (Beta), P.1 (Gamma)	Unclear	Detection rate of antigen/antibody tests to detect these three variants were reported to be >90%	[317]

specific recognition of a unique virus genome mutation [155]. In terms of the assay sensitivity and specificity, the use of the CRISPR/Cas-based detection technique was also shown to provide superior sensitivity and specificity in detecting the SARS-CoV-2 variant (~100%), making it another alternative tool to screen for virus mutation [315].

Although some published protocol has suggested that protein-based tests like viral antigen and the serological test could be potentially used to detect SARS-CoV-2 variant (Table 19), both these tests could not distinguish between different VOI and VOC and are said to have poorer sensitivity and specificity [296]. A study has previously reported the use of virus antigen test to try to identify different SARS-CoV-2 variants and the LOD was reported to be 1.7–6.6 $\times$ 10^7 RNA copies in 1 mL [316]. The test sensitivity and specificity in confirming the identity of different SARS-CoV-2 variants, however, was not described further by the authors [316]. In another study [317], it was demonstrated that antigen and the antibody-based test were sensitive (>80%) to identify three SARS-CoV-2 variants, namely, Alpha (B.1.1.7), Beta (B.1.351), and Gamma (P.1). This suggested that protein-based tests could be helpful to aid in screening identity of virus variants in a shorter test time [317]. However, sequence analysis is still recommended to be done to further confirm the screening test results [296].

5. Conclusion

This review summarizes the various approaches and methods, which could be used to confirm the diagnosis of both the MERS-CoV and SARS-CoV-2 infections. To date, qRT-PCR and viral nucleic acid sequencing serve as the two most commonly used and accurate diagnostic tests to confirm MERS-CoV and SARS-CoV-2 cases. Protein-based detection tests like viral serological tests could aid in determining whether a patient has past or recent acute infection by the coronavirus, but the test might be less sensitive to detect the presence of the antibodies towards the viruses if the individual is in the early stage of infection, or has long recovered from the infection. Virus cell culture and virus neutralization tests could be alternative tests to confirm the coronaviruses infections, but both of these tests require highly trained personnel to perform the tests, and that the test results take a longer time to be available. Clinical evaluation and radiological assessment of the thoracic cavity of the patients might help predict MERS-CoV and SARS-CoV-2 infections. However, confirmatory tests are still required. Compared to MERS-CoV which is now causing sporadic transmission worldwide, SARS-CoV-2 still causes a severe global pandemic crisis. By far qRT-PCR is still the most recommended test to screen for the transmission of SARS-CoV-2 worldwide while rapid antigen tests and serological tests like ELISA could help in screening for the presence of the virus when the qRT-PCR service is unavailable, especially in the remote regions. It is hoped that with the continuous development in molecular diagnostic technology, cheaper, faster, and highly sensitive diagnostic kits could be made available to allow massive screening of the coronavirus worldwide to help block the transmission chain, and thus, the world could be able to return to the normal state before the Covid-19 pandemic started.

Authors' contributions

Swee Keong Yeap, Chean Yeah Yong, Wan Yong Ho and Stephanie Y. L. Ng conceptualized the idea, developed the structure of the article and

guided the selection of references; Zhi Xiong Chong, Winnie Pui Pui Liew, Hui Kian Ong, Chong Seng Shit, Swee Keong Yeap, Chean Yeah Yong, Wan Yong Ho and Stephanie Y.L. Ng prepared, wrote and edited the draft; Zhi Xiong Chong prepared the figures/Tables. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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