Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Review article

Molecular diagnosis of COVID-19 in different biologic matrix, their diagnostic validity and clinical relevance: A systematic review

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ABSTRACT

Due to COVID 19 outbreak many studies are being conducted for therapeutic strategies and vaccines but detection methods play an important role in the containment of the disease. Hence, this systematic review aims to evaluate the effectiveness of the molecular detection techniques in COVID-19. For framing the systematic review 6 literature databases (PubMed, EMBASE, OVID, Web of Science, Scopus and Google Scholar) were searched for relevant studies and articles were screened for relevant content till 25th April 2020. Observations from this systematic review reveal the utility of RT-PCR with serological testing as one such method cannot correlate with accurate results. Availability of point of care devices do not conform to sensitivity and specificity in comparison to the conventional methods due to lack of clinical investigations. Pivotal aim of molecular and serological research is the development of detection methods that can support the clinical decision making of patients suspected with SARS-CoV-2. However, none of the methods were 100% sensitive and specific; hence additional studies are required to overcome the challenges addressed here. We hope that the present article with its observations and suggestions will assist the researchers to realize this vision in future.

1. Introduction

SARS-CoV-2 is a betaCoV whose ss RNA genome contains 29,891 nucleotides, encoding for 9860 amino acids [1]. Genomic analyses suggest its evolution from a strain found in bats with +ssRNA of approximately 30 kb in length; the largest known RNA viruses [2]. identified the genomic sequence of SARS CoV-2 and sequence homology of 82% with SARS-CoV [3]. 2019 Novel human pathogenic coronavirus (SARS-CoV-2) encompasses four structural proteins i.e. Spike, Envelop, Membrane and Nucleocapsid (S, E, M, and NC) protein. In one of the studies by Gralinski et al. [4], it was confirmed that SARS-CoV-2 uses the ACE2 (Angiotensin-converting enzyme 2) cell receptor for its entry into the host cell [5].

The envelope (E) protein being the smallest protein mediates the assembly, envelope formation, budding, and release of the complete virus particle and also promotes the pathogenesis of SARS-CoV [6,7] by forming ion channels [8] The nucleocapsid (NC) protein binds with RNA by interacting with the +sense ss RNA molecule [9,10] and also regulates the virus replication, transcription, translation, assembly, release, and pathogenesis [11,12]. Due to this reversible attachment, it acts as a receptor destroying enzyme [13,14]. SARS-CoV-2 genome encodes for 16 non-structural proteins (nsp1-16) located on the ORF of polyprotein 1a/1ab with different functions altogether [15]. Nsp5 (3CLpro) is considered as the main protease and the other protease is papain-like protease (nsp3), both of them cleave the pp1a and pp1b to form 16 nsp's [16]. Therefore, all these nsp's help in viral replication, transcription, RNA processing and escape from host immune response [17].

Currently, the method used for diagnosing SARS-CoV-2 is RT-PCR but its sensitivity is low in the early stages of the COVID-19 infection.

Abbreviations: ICTV, International committee on taxonomy of viruses; ACE2, angiotensin converting enzyme 2; LOD, limit of detection; LAMP, loop-mediated isothermal amplification; VIVALDI, veterinary validation of point-of-care diagnostic instrument; LSPR, localized surface plasmon resonance; FET, field effect transistor

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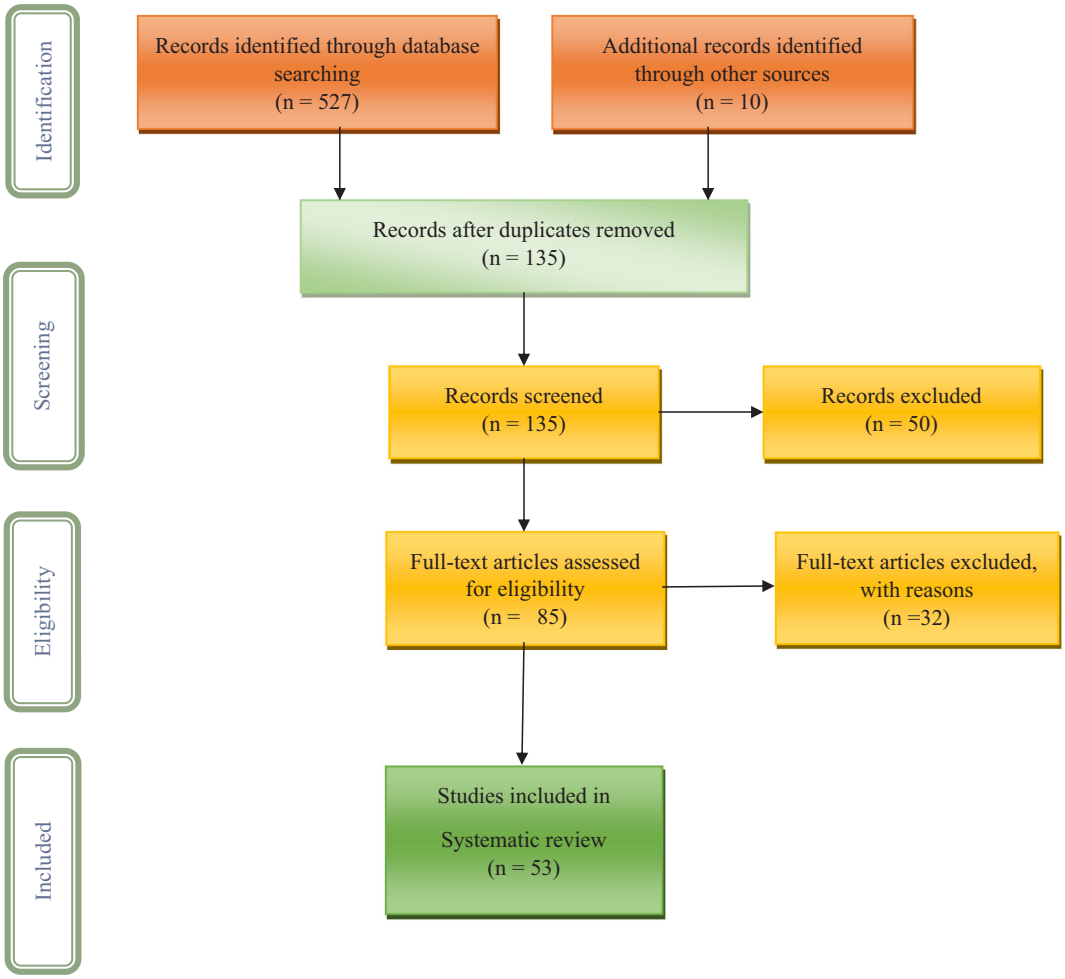

Fig. 1. Schematic flow diagram to show selection criteria for systematic review.

Table 1
Advantages and disadvantages of the molecular detection methods for COVID-19.

Diagnostic test	Advantages	Disadvantages
RT-PCR [74–76]	Independent diagnostic because it has high specificity.	Risk of providing false negative and false positive results and has low sensitivity.
		Collection of samples specifically the bronchoalveolar lavage (BALF) becomes difficult as it requires suction tool and an expert operator and is also painful for the patient.
Serological Testing (IgG and IgM based assay) [77]	Fast, robust and easy to perform	Inability to detect the presence in early stage of disease due to late generation of antibodies after exposure.
	May be able to detect the mild infected patients to prevent the spread of the infection and can be useful in case of community-based surveillance.	Cross-reactivity as it impacts the sensitivity and specificity.
Point of Care [78,79]	Quick method thus putting less strain on healthcare workers	It needs high level technical expertise and are expensive thus prohibiting widespread use.
		Currently available POCT are questionable for clinical purposes and cannot be substituted for other molecular tests.

Other modified techniques have also been used as discussed [18] which can give results in less than 5 min and is highly sensitive than RT-PCR [19,20]. Many molecular-based detection methods have been made available since the outbreak and because of lack of time, their effectiveness cannot be evaluated. To our knowledge this is the first systematic review evaluating and interpreting the effectiveness of different detection methods for COVID-19. The present article with its observations and suggestions will provide the researchers and scientists the scientific evidence on the potential of the most appropriate detection method to be used for the diagnosis of symptomatic as well as asymptomatic COVID-19 patients.

2. Methods

We searched 6 literature databases (PubMed, EMBASE, OVID, Web of Science, Scopus and Google Scholar) for relevant studies till 25th April 2020 using keywords detection, diagnostic kit, identification kit, test kit, SARS2CoV, SARS Coronavirus, Coronavirus 2, COVID-19,

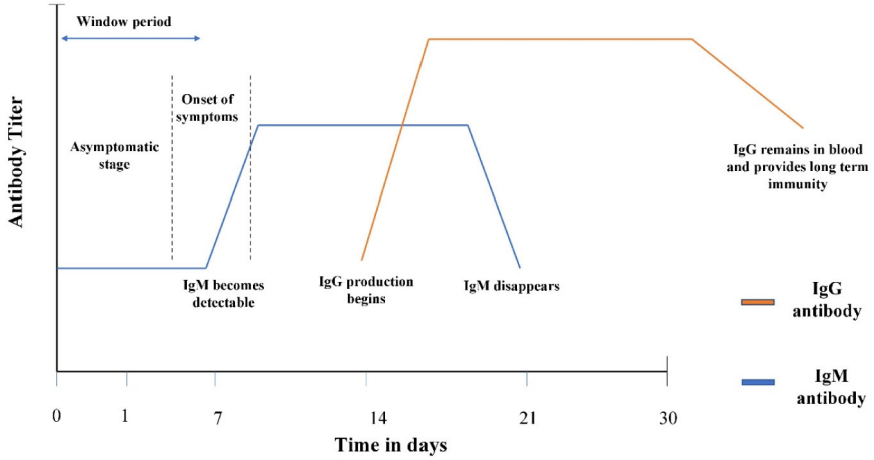

Fig. 2. Level of antibodies in SARS-CoV-2.

nCoV-19, Novel Coronavirus. The details are given in Prisma Chart (Fig. 1).

2.1. Inclusion and exclusion criteria

Studies included reports different diagnostic kits/test kits/point of care diagnostic devices or kits/using blood/serum/sputum or any biological fluid for the diagnosis of COVID-19. Articles with radiological or clinical diagnostic modules were excluded and all studies were included irrespective of the language. Google translator was used for articles with different language and if not understandable the article was excluded.

2.2. Data extraction and analysis

Endnote was used for article extraction and removal of duplication. Independent screening of titles/abstracts was done by SM, GB, and PS using the predefined inclusion/exclusion criteria and further evaluation was performed for possible inclusion. AP and BM were consulted for any discrepancies. SM and GB independently extracted the data using Cochrane data extraction form and authors were contacted for providing necessary information.

3. Results

We identified 537 studies through database searches. After reviewing the title and abstracts, we excluded 452 studies that were not relevant, leaving 85 studies for full-text evaluation. Of these, 53 studies fulfilling predefined inclusion/exclusion criteria were finally included in the current systematic review.

3.1. RT-PCR based detection methods

RT-PCR protocol for COVID-19 testing was established and validated in the absence of virus isolates. These assays target structural and non-structural protein of the virus or even the viral nucleic acid [17].

3.1.1. RT-PCR based assay targeting Enveloped (E) and RNA dependent RNA polymerase (RdRp) protein

Corman et al. conducted initial studies for assay evaluation [21] using nasal, throat swabs and fecal samples and isolation of RNA was performed with MagNA Pure 96 system with Platinum Taq Polymerase where E gene was selected as screening tool while RdRb recommended for confirmation. In regards of analytical sensitivity, it was 5.2 copies and 3.8 copies per reaction at 95% detection probability for E gene and RdRp gene respectively. Other labs also showed equivalent results and LOD for RdRp gene and E gene was 3.6 copies and 3.9 copies per reaction with no cross-reactivity with other viruses. Clinically tested on 297 samples using E and RdRp gene assay already infected with other respiratory viruses indicating no false positive outcomes. Cordes et al. [22] developed an automatic Hologic Panther Fusion (PF) system processing the sample in 3.5 h targeting E gene and RdRp using similar RT-PCR protocol and the results were similar to Corman et al.

Similarly, first manufacturer-independent fully automated system of Cobas 6800 was developed by Poljak et al. [23] using Corman et al. [21] RT-PCR protocol as comparator. For In-house validation panel 217 samples (2 samples were excluded) were tested showing overall 98.1% agreement (211/215; 95% CI: 95.0–99.4%), positive percent agreement of 95.2% (60/63; 95% CI: 85.8–98.8%), negative percent agreement of 99.3% (151/152; 95% CI: 95.8–100.0%) and for head-to-head analysis using 502 samples of similar individuals excluding one sample. There was 99.6% (499/501;95% CI: 98.4–99.9%) overall agreement, positive percent agreement of 100.0% (63/63; 95% CI: 92.8–100.0%), negative percent agreement of 99.5% (436/438; 95% CI: 98.2–99.9%). Observations are suggestive of modest increase of analytical sensitivity of Cobas over the LightMix approach. Further, validation demonstrated E gene for diagnosis but RdRb gene targeting was recommended for confirmation.

3.1.2. RT-PCR based assay targeting E-gene

Pfefferl et al. [24] developed an automated cobas 6800 system for the detection of SARS-CoV-2 using the open channel (utility channel) targeting the E gene. The primers used had modifications in their penultimate base with 2′-*O*-methyl bases for preventing primer dimers formation using in vitro-transcribed RNA (IVT RNA) as a positive control for E gene. Assay was performed on swab samples with LoD of 689.3 copies/mL with 275.72 copies per reaction at 95% detection probability which was roughly in line with the results published by Corman et al. [21]. No cross-reaction was observed with an additional advantage of the handling large number of samples to keep pace with changing demand.

3.1.3. RT-PCR based assay targeting spike protein-encoding gene (S gene)

To-Wang et al. [25] performed In-house 1-step real-time reverse transcription-quantitative polymerase chain reaction (RT-qPCR) assay on saliva specimens of 12 patients against S gene using NucliSENSeasyMAG (BioMerieux) for RNA extraction in a LightCycler 480 Real-Time PCR System (Roche). Specimen collection was done 2 days after hospitalization with highest viral load in earliest available specimens for 5 patients (83.3%) with additional shedding after 11 days of hospitalization for 1 patient.

3.1.4. RT-PCR based assay targeting ORF 1ab (Open Reading Frame)

Wang et al. [26] detected SARS-CoV-2 using qRT-PCR method targeting 1*ab* ORF (Open Reading Frame) recruiting 1070 specimens. Observations suggested higher sensitivity of BALF (Bronchoalveolar Lavage Fluid) with a positive detection rate of 95%, followed by sputum (72%), nasal swab (63%), bronchoscopy (fibrobronchoscopy brush biopsy) (46%), pharyngeal swabs (32%), feces (29%), and blood (1%). Even none of the samples came positive for SARS-CoV-2 for 72 urine specimens, none of the samples came positive for SARS-CoV-2. Above studies suggested similarity of the results with targeting of single genes using RT-PCR protocol with only difference in primer designing.

3.1.5. RT-PCR based assay targeting E-gene and spike protein-encoding gene (S gene)

Amrane et al. [27] utilized two different RT-PCR systems with a hydrolysis probe and the LightCycler Multiplex RNA Virus Master kit (Roche Diagnostics®, Mannheim, Germany) on 280 suspected COVID-19 patients using sputum and nasopharyngeal samples against E-gene and Spike protein-encoding gene using synthetic RNA as a positive control. All samples tested negative as the results were obtained approximately within 3 h of arrival of the patient samples at the laboratory. Lagier et al. [28] presented similar negative results on 337 French natives (tested on day 0 and 5) performing RT-PCR with QuantiNova SYBR Green RT-PCR kit (Qiagen) on nasal and oropharyngeal samples against similar genes and using same probes as by Amrane et al.

These studies specifically focused to prevent the transmission by isolating the confirmed cases of suspected individuals with a travel history.

3.1.6. RT-PCR based assay targeting ORF1ab (Open Reading Frame) and Nucleocapsid gene (N gene)

Chu et al. [29] conducted Two monoplex real-time RT-PCR assays targeting the ORF1b and N gene regions of 2019-nCoV. The amplification efficiencies of ORF1b and N gene assays were 99.6% and 95.4%, respectively and for clinical sample detection two suspected patients were tested positive via this assay. Liu et al. [30], in his study tested 4880 cases with quantitative RT-PCR (qRT-PCR) on respiratory tract samples. The positive rate was 38.42% (1875) in a total of 4880 specimens, out of which 39.80% were positive for Nucleoplasmid Protein and 40.98% for ORF1ab. There was a poor positive rate for nasal and pharyngeal swabs (38.25%) in contrast to 100% positive rate for ORF1ab in bronchoalveolar lavage fluid (BLF).

Yu et al. [31] made a comparison of droplet digital PCR (ddPCR) with the conventional RT-PCR targeting ORF1ab and N gene on 323 samples from 72 confirmed patients using swabs, throat swabs, sputum, blood, and urine as the samples. Results of RT-PCR demonstrated 161 samples negative, 95 positive and 67 single-gene positives. The ddPCR confirmed positive for 95 positive samples with high correlation of RT-PCR Ct value with copy number determined by ddPCR. Among the 67 single-gene positive samples, 26 (38.8%) were negative in ddPCR and 41 (61.2%) were positive with copy numbers ranging from 11.1–123.2 copies/test. Among the 161 negative samples identified by RT-PCR, 157 (97.5%) samples were negative by ddPCR, and 4 samples were positive with the copy number ranging between 11.3 copies/test and 20.7 copies/test. This showed the reliability and accuracy of both the methods with high viral loads and better performance of ddPCR with low viral loads. Limitations of the study included absence of matched controls and limited sample size.

3.1.7. RT-PCR based assay targeting ORF1ab, Nucleocapsid protein (N) and Enveloped (E) protein

Wang et al. [32] compared LOD's of 6 different kits approved by China National Medical Products Administration (NMPA) using RT-ddPCR. Clinical Laboratory Standards Institute guidelines indicated (CLSI), the LOD as 95% detection rate for positive results of each kit. The LoDs of four of the kits were 484 copies/mL (Liferiver, Huada, DAAN, Sansure) whereas the LoD of BioGerm was 968 copies/mL and for GeneoDx it was 7744 copies/mL, giving a maximum 16-fold difference.

The results of the study done by Chu et al. suggested targeting of N gene as diagnostic measure and ORF1ab as the confirmatory targeting. Hence studies targeting two or more genes had better result profile compared to single gene alone. Hence, molecular testing was set as the gold standard diagnosing SARS-CoV-2 and E and RdRb gene indicating higher analytical sensitivity compared to combination of other genes such as N and ORF1ab gene.

3.1.8. Comparison between different clinical specimens via RT-PCR

To compare the presence of the nCoV-19 in different clinical specimens Ye et al. [33] recruited two groups of patients with 46 patients in 1st group and 45 in 2nd group. In 1st group, 25 (54.3%) tested positive by RT-PCR on throat swabs and 36.9% (17/46) positive on lingual swabs; lingual swabs positive patients were also positive on throat swabs. Among the 2nd group (45 patients), 48.9% (22/45) were infected with SARS-CoV-2 detected with RT-PCR where positive outcome on throat swabs was 33.3% (15/45) while it was 35.6% (16/45) on lingual swabs. In a total of 91 patients, the positive outcomes via throat swabs (44.0%) were higher compared to lingual swabs (36.3%) and yet in another study by Wu et al. [34], out of 132 patients the positive rate for nasopharyngeal swab was 38.13%, 48.68% for sputum, 3.03% for blood, 9.83% for feces and 10% for anal swab. Fecal anal samples tested positive for specimen's negative for Nasopharyngeal swab (NPS) and sputum indicating earlier virus clearance from respiratory tract than digestive tract. Similar results on fecal samples were observed by Zhang et al. [35] indicating shedding of virus in stool as well as in body fluids hence making testing mandatory of different body samples thus decreasing false-negative results along with more sensitivity in the carriers as well as severely ill patients.

Wang et al. [36] with 353 patients suggested the suitability of NPS over oropharyngeal swab (OPS) and Lin et al. [37], in his study with 52 patients showed positive reaction for 23 throat swab (44.2%) and negative for 29 (55.8%) in contrast to 40 (76.9%) positives for sputum and 12 negative (23.1%) indicating the positive score almost 2-fold that of throat swabs. Guo et al. [38] compared nasopharyngeal swabs (NP) and throat washings in 11(6 hospitalized and 5 discharged patients) targeting Nucleocapsid protein and ORF1ab gene. 14 out of 24 paired throat washings and NP swabs showed negative results, including 5 in discharged patients and 19 in hospitalized at a median of 53 days after symptom onset (range: 48–57 days) with the exception of other 5 paired samples as they got inconsistent results. According to the Chi-squared test, rate of positive outcomes of throat washings was much higher compared to NP swabs ($P = 0.031$).

Two studies by Williams et al. [39] and Azzi et al. [40] was conducted using saliva where in former all 25 saliva samples confirmed the presence of the virus even after 4 days of sample collection 8 patients showed consistent results. For the latter study (622 patients recruited) 39 (6.3%; 95% confidence interval [CI] 4.6%–8.5%) were positive for Nasopharyngeal swabs (NPS) and 33 (84.6%; 95% CI 70.0%–93.1%) positive for saliva specimens. For specificity assessment 1 sample was saliva positive out of 50 PCR negative swabs indicating difference in the quality of NPS collection.

Xiao et al. [41] demonstrated SARS-CoV-2 conversion characteristics of nucleic acid analyzing throat swab or deep nasal cavity swab samples of 70 patients. Results showed 15 (21.4%) patients who were testing consecutively two times negative came out to be positive a third time as detected by RT-PCR. Additionally, a patient tested positive 45 days after symptoms onset suggesting longer monitoring time for certain group of COVID-19 patients. Similar to these results, Pan et al. [42] demonstrated the effect of thermal inactivation on viral nucleic acid testing (NAT) recruiting 23 confirmed patients consisting of 19 throat swabs, 2 sputum samples, and 3 stool samples with inactivation done through incubating in water bath at 56 °C for 30 min. 4 clinical

samples were also being tested by RT-PCR for comparison. No significant effect of thermal inactivation was seen for specimens with high viral loads but Ct values were quite high (ranging from 33.37 to 36.89) for specimens (7 of 15 samples) with low viral loads thus indicating false-negative results. Also, a comparison was made between Guanidinium-based buffer (GL) (solution for specimen preservation which also inactivates the virus) and thermal inactivation (TI) and it was observed that Ct values for TI group were (mean 36.48 ± SD 1.48) and for GL group it was (mean 35.40 ± SD 1.33).

3.1.9. Time-dependent testing via RT-PCR

Roxby et al. [43] reported results of testing done at day 1st and day 7th. Among 142 residents 5 who tested positive on day 1 were negative for 1 and positive for 2 residents on day 7th while other two not re-tested. One resident who was negative on day 1 tested positive on day 7th.

Analysis of different clinical samples advocates the use of throat swabs as they provided the most positive test results. Saliva with lower sensitivity compared to NPS can still be used in clinical settings with low resources conserving the latter for high clinical index suspicious patients. Overall, the above studies suggested the use of all available biological samples for the confirmatory testing and removal of bias.

3.2. Modified qRT-PCR technique protocols

The primary goal of developing these modified protocols is to determine the negative rather than the positive cases so that a negative person can be allowed to work or study without worry and a positive person can be recommended for further diagnosis with certified diagnostic kits.

Hence Won et al. [44] developed a low-cost, safe and efficient protocol to detect SARS-CoV-2, where they analyzed the pharyngeal swab specimen of 14 volunteers by a “self-collection procedure” and RNA extraction (TriZol based method) was done. Sensitivity of 1–10 virus particles was observed and the results were obtained in less than 4 h. Chan et al. [45] developed a novel qRT-PCR with high sensitivity targeting three viral genes, RNA-dependent RNA polymerase (RdRp)/helicase (Hel), nucleocapsid (N) protein, spike (S) protein and compared it with RdRp-P2 assay. 273 specimens (respiratory tract and non-respiratory tract) from 15 COVID patients were evaluated using NucliSENSeasyMAG extraction system (BioMerieux, Marcy136 l'Étoile, France) for RNA extraction. No cross-reactivity was present and also observed 77 specimens to be positive out of 273 using both assays (COVID-19-RdRp/Hel and RdRp-P2) compared to 42 positive specimens which tested negative using RdRp-P2 assay. This comparison highlighted the high specificity and sensitivity of COVID-19-RdRp/Hel assay.

Yip et al. [46] in his study used in-house program called Golay-MetaMiner, targeting the longest and previously untargeted nsp2 region. Sensitivity in terms of LOD was 1.8$TCID_{50}$/mL with no cross-reactivity. For diagnostic evaluation 59 clinical specimens from 14 confirmed cases were used, which demonstrated 100% concordance with the results obtained from previously used RdRp/Helicase assays (23 positives and 36 negatives). Nalla et al. [47] compared three different extraction methods using seven different primer/probes sets with one assay comparing the performance of SARS-CoV-2 detection assays. Two separate systems were used i.e. MagNA Pure LC 2.0 and the MagNA Pure 96 (Roche Lifesciences) for the extraction of RNA. Three additional probes were also designed referred to as Corman N-gene, RdRp, and E-gene primer/probe sets. AgPath-ID One-Step RT-PCR kit (Life Technologies) was used to perform the RT-PCR along with BGI RT-PCR detection kit (BGI). The tests gave no false-positive results showing its specificity in contrast to the variability in the sensitivities also revealing the sensitivity using CDC N2 and Corman E-gene primer/probe sets and ability to detect all 10 positive clinical samples of SARS-CoV-2.

Yet in another study by Moran et al. [48] compared two assays Roche cobas specific for ORF1ab and part of E-gene and Cepheid Xpert Xpress specific for N2 region of the N gene. Out of 8 nasal and 95 NPS (total 103 samples) 42 tested positive and 60 negatives on both systems with an agreement of 99% but the study was limited by small sample size and cross-reactivity with other viruses, but if both assays could be used together it can maximize the COVID-19 testing. Another modified protocol was an optimized Triplex RT-PCR by Waggoner et al. [49] targeting three proteins N, E and RNase P and the LOD came out to be 45 copies/μl. Results demonstrated 100% agreement with Center for Disease Control and Prevention (CDC) and Corman Protocol when tested on 27 patients with suspected symptomatic SARS-CoV-2 infection.

Therefore, these modified and improved assays may help in the rapid, efficient ultra-specific and large-scale screening of SARS-CoV-2 infections.

3.3. IgG-IgM combined antibody test of SARS-CoV-2

RT-PCR being the gold standard in diagnosis of SARS-CoV-2 still poses many limitations due to reporting of false-negative results. Therefore, requiring an accurate, rapid method which can quickly lead to identification of infected patients and asymptomatic carriers for further prevention of viral transmission and assuring timely treatment of patients [50].

3.3.1. IgG-IgM targeting Spike (S) Protein

Li et al. [50], in his study used pharyngeal and sputum samples for detecting IgG and IgM. There were total of 525 samples, 128 (non-SARS COV 2 infected) clinical negative samples and 397 positive clinical samples (SARS-COV 2 infected) using recombinant antigen (MK201027) which is a receptor-binding domain of SARS-CoV-2 Spike protein. This antigen has a specific binding to SARS-CoV-2 antibodies (including IgM as well as IgG) having conjugation with sprayed on conjugation pads and colloidal gold nanoparticles.

Sensitivity and specificity for the test were 88.66% and 90.63%, respectively using un-inactivated vein blood. The comparison was also made between serum, plasma and fingerstick blood of the venous blood of 7 patients and 3 healthy volunteers out of which 3 had only IgM positive and 4 both IgG and IgM positive. There was 100% consistency among the corresponding blood samples.

Limited time and no complete information on the duration of the infectivity of the patient w the limitations of the study. Even differential level of antibodies was not compared in different stages of the infection. On the contrary the advantages of this test were its less time-consuming nature and that it does not require equipment and convenient as fingerstick blood can also be used. It can screen asymptomatic SARS-CoV-2 carriers as well.

3.3.2. IgG-IgM targeting Nucleocapsid (N) Protein

Guo et al. [51] developed indirect ELISA protocol for detecting IgM, IgA, and IgG antibodies against SARS-CoV-2 using purified rNPs (recombinant Nucleocapsid protein) as coating antigens with testing on 208 blood samples from 2 cohorts. In 1st cohort (43 blood samples called as “confirmed cases” and 58 called as “probable cases”) and in 2nd cohort (39 confirmed cases) with 285 samples were used as controls.

Collection period was: 41 samples (1–7 days), 84 samples (8–14 days), 83 (> 14 days) post symptom onset (PSO) with 188 and 194 positive numbers of IgM and IgA. For CC and PC, the positive rate of IgM was 75.6% and 93.1% with higher detection efficiency of by IgM ELISA compared to qPCR after 5.5 days of symptom onset. The study also showed significantly increased positive detection rate (98.6%) using IgM ELISA assay with PCR compared to a single qPCR test (51.9%).

Xiang et al. [52] also used ELISA based detection against recombinant Nucleocapsid protein (rN) in 66 confirmed cases of COVID-

19 at 3–40 days after the onset of the symptoms and results were evaluated on the basis of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and consistency rate. For IgM it was observed to be 77.3% (51/66), 100%, 100%, 80.0%, and 88.1% and for IgG it was 83.3.3% (55/66), 95.0%, 94.8%, 83.8%, and 88.9 and similar to other studies the trend of the occurrence of antibodies were same, hence this testing can be an adjunct to viral nucleic acid testing by RT-PCR.

3.3.3. IgM and IgG based assay targeting Nucleocapsid (N) and Spike (S) protein

Gao et al. [53] collected 37 serum samples from 22 confirmed cases of SARS-CoV-2 where 10 came from the early stage (1 to 7 days after infection onset), 13 from the middle stage (8 to 14 days after infection onset), and 14 from the late-stage (14 to 24 after infection onset). Antibody levels were measured using ELISA, chemiluminescent immunoassay (CLIA) and gold immunochromatographic assay (GICA). Results indicated 6/10, 7/13, and 11/14 positive for IgM for early stage, middle and the late-stage respectively and for IgG, it was 5/10 and 10/13 and 14/14. GICA had higher positive detection rate for IgM compared to ELISA which had higher positive detection rate for IgG. Hence, a combination of GICA and ELISA might be an effective way for early screening and diagnosis for 2019-nCoV infection.

Similarly, Zhong et al. [54] also developed serological testing detecting IgM and IgG against spike (S) and nucleocapsid (N) proteins via ELISA and chemiluminescence in 47 patients and 300 healthy controls. The specificity and sensitivity for recombinant nucleocapsid protein (rN) based IgG was 99.7% and 97.9% and for recombinant spike protein (rS) based IgG the values were 85.7% and 95.7%. Likewise, rN-based specificity and sensitivity of IgM were 99.7% and 97.9% and for rS-based, it was 89.1% and 97.0%, respectively. In comparison to ELISA, in Chemiluminescence sensitivity of 95.6% and specificity of 96.6% was observed for IgG and for IgM it was 97.7% and 95.2%. So, to sum up the study both the methods displayed good consistency demonstrating better sensitivity of rN based IgG than rS based IgG via ELISA for differentiating the patients from the controls. In contrast to this, differentiation could be done between positive patients from the controls based on rN-based and rS-based IgM making it a good tool to be used with the nucleic acid test.

In another study by Liu et al. [55] where pharyngeal swabs of 214 patients with a median of 15 days post disease onset (d.p.o) (range, 0–55 days) were recruited. Out of 214, the positive cases identified by rN-based IgM and IgG ELISAs were 146 (68.2%) and 150 (70.1%). The number of positive results were 165 (77.1%), 159 (74.3%), and 176 (82.2%) when detected with rS-based ELISA for IgM, IgG, and IgM and/or IgG. The time-dependent analysis was also done where patients were divided into 7 groups on the basis of days from onset of the disease to serum collection (0–5, 6–10, 11–15, 16–20, 21–30, 31–35, and > 35 d.p.o.). For rN based ELISA a lower positive rate for IgM and IgG was seen at 0–5 d.p.o. and 6–10 d.p.o. and a higher positive rate of IgM was seen compared to IgG at 6–10 d.p.o. and decreased after 35 d.p.o. demonstrating the rise in IgG concentrations with dropping levels of IgM. The positive rate was 88.9% at 11–15 d.p.o., for IgM and/or IgG and at later stages it became more than 90%. A similar trend was seen for IgM and IgG positive rates for rS based ELISA's, except > 35 d.p.o., owing to the higher sensitivity of rS-based IgM detection (77.1%) than IgG detection (74.3%). Therefore, combined use of rN- and rS-based ELISAs was not suggested; however, if an ELISA kit coated with a cocktail of N and S polypeptides can show better results, but needs further evaluation.

For the investigation of the analytical value of serological diagnosis and to study the active variability of SARS-CoV-2 antibodies Jin et al. [56] studied 43 patients retrospectively and chemiluminescence was used for the measurement of IgM and IgG. Specificity for both IgG and IgM was found to be than 90% in comparison to molecular detection. Further a higher positive rate of IgG (88.9 was seen than IgM (48.1%) and additionally increased viral antibodies were noticed in early stages of the disease. Thus, suggesting non-exclusion of the disease at an initial stage even when serological testing gives negative result. Also, there was initial increasing trend in IgM positive rate which then decreased, in contrast to increased and stable IgG positive rate as time went on.

Antibody testing was mainly a time dependent mechanism where higher levels of IgM were noticed at initial stages and IgG at later stages. Studies also demonstrated an elevated sensitive detection with rN based IgG compared to rS based IgG in contrast to IgM where rN and rS antigens both can be used for the discrimination of the patients with the controls. Certain limitations include false-negative results owing to time of sampling and secondly, cross-reactivity can be seen with other respiratory viruses and other molecules such as interferons, rheumatoid factor, and non-specific IgM can produce false-positive results. Hence it would be appropriate targeting both N and S protein using combination of the assays for the verification of results.

3.3.4. Spike (S) protein and Nucleocapsid (N) protein targeting using both RT-PCR and IgG and IgM based assay

Zhang et al. [57] analyzed oral swabs, anal swabs and blood specimens using qPCR targeting viral spike (S) gene and serological tests against nucleocapsid (N) protein. Through RT-PCR 8 (53.3%) out 15 patients were positive over oral swabs, 3 were serum positives (20%), 4 anal swabs positive (26.7%) and 6 blood positives (40%). In second investigation, the patients targeted were the ones who received 10 days medical treatment after admission. IgM and IgG titers were proportionally low or undetectable at day 0 and an increase in the viral load at day 5 was observed. The positive rate of IgM increased from 50% (8/16) to 81% (13/16), whereas for IgG it increased from 81% (13/16) to 100% (16/16). These results were in contrast to proportionally low detection positive rates from the molecular test.

Yunbao Pan et al. [58] compared serological immunochromatographic technique with RT-PCR where they performed colloidal gold-based immunochromatographic strip assay (ICG). 134 samples from 105 patients were collected, out of which 87 samples from 67 cases confirmed by RT-PCR were analyzed. The disease stage was divided as early (1–7 days from onset), intermediate stage, (8–14 days) and late-stage (more than 15 days). In early stage IgM positive rate was 11.1% which raised to 78.6% and 74.2% in intermediate and late stages. In confirmed patients the positive rate of IgG was 3.6% in early, 57.1% in intermediate and 96.8% in late stage. These results collectively suggest that IgM is produced early and IgG at later stages with the progression of the disease. Further 39 nucleic acid samples were tested for efficacy testing of ICG, where 9 were positive to IgM and 15 were positive to IgG also confirming whole blood to be in excellent agreement compared to plasma with a kappa coefficient value of 0.93 (95% CI, 0.80–1.06).

3.3.5. Comparison between RT-PCR based testing and Antibody based testing

Wu et al. [59], in their only study provided the data comparing RT-PCR and antibody-based testing, recruiting 1021 resuming patients and 381 hospitalized patients. No patient was tested positive among the 1021 patients via RT-PCR and only one female was NAT, IgM and IgG positive among 381 patients. On the contrary 39 out of 380 were IgG positive but IgM and Nucleic acid testing (NAT) negative, and from the resuming group 98 IgG positive and IgM and NAT negative which suggested recovered asymptomatic patients as they had no history of COVID-19.

3.4. Point of care (PoC) devices

Various limitations restrict the use of RT-based tests and serological testing for COVID-19 as they are quite laborious and time-consuming. Therefore, to effectively combat its transmission population-scale testing is required that can enable the rapid identification process of infected individuals.

Jonathan et al. [60] proposed a novel LAMP Seq protocol allowing for population-scale testing using sample-specific barcodes. This technique gives the advantage of eliminating RNA extraction, provides sterilization before shipment and allows for logistics for a large number of samples. Nguyen et al. [61], in his study cited Veterinary validation of point-of-care diagnostic instrument (VIVALDI) project for using LAMP as PoC. Lateral flow strip (LFS) developed by BioMedomics based on the principle known as COVID-19 IgM/IgG Rapid whose sensitivity is 88.66% lower than LAMP based assays. Therefore, combining LAMP and LFS into one single device could be an excellent candidate for PoC testing of novel coronavirus.

Clinically Lateral Flow Immunoassay (LFIA) was validated by Chen et al. [62] using lanthanide-doped polysterene nanoparticles (LNPs) to IgG antibodies against nucleocapsid (N) protein in human serum. Only 1 sample came to be positive out of 12 negatives from RT-PCR suggesting the false-negative outcomes of PCR. CRISPR-Cas-12 based lateral flow assay mentioned by Broughton et al. [63] which used extracted RNA known as SARS-CoV-2 DNA Endonuclease-Targeted CRISPR Trans Reporter (DETECTR) and simultaneously uses RT-LAMP followed by Cas12 detection. The LOD was10 copies per μl reaction with 95% positive predictive agreement and 100% negative predictive agreement compared to CDC RT-PCR assay. Similarly, Park et al. [64] designed and evaluated one-step reverse transcription LAMP (RT-LAMP) method giving a limit of detection (LOD) of 100 copies per reaction.

Clinical evaluation of RT-LAMP was done by Lu et al. [65] where six sets of LAMP primers (4, 1, and 1 in N, S and RdRp genes) were designed. 17 tested positive out of 24 with RT-PCR as well as using RT-LAMP assay demonstrating 100% consistency. Similarly, Yan et al. [66] in his study on 34 patients and analyzing 130 specimens targeting spike (S) gene and ORF1ab gene. Sensitivity of 100% (95% CI 92.3% - 100%), specificity 100% (95% CI 93.7% - 100%) comparable to RT-PCR reported previously. Further used by Yu et al. [67] using LAMP based method iLACO (isothermal Lamp based method for COVID-19) with 89.9% (223/248) positive and 25 negative results. Baek et al. [68] used 154 clinical samples (nasal swabs) showing a sensitivity and specificity of 100% and 98.70% suggesting the use of primers for early identification of SARS-CoV-2.

Among these POC devices two studies by Harrington et al. [69] and Rhoads et al. [70] comparing two devices: ID NOW COVID-19 (IDNCOV) assay and Abbott 33 Real Time SARS-CoV-2 (ACOV) assay on 524 nasal swabs giving a 75% positive agreement (67.74, 80.67) and 99% negative agreement (97.64, 99.89). The latter study compared the ID NOW and Simplexa with modified CDC method where 96 clinical samples were tested where the virus was detected in all samples in CDC method whereas for ID NOW and Simplexa were 90 ((PPA 94% [CI 87–98%])and 92 (PPA 42 96% [CI 90–99%]).

Qiu et al. [71] and Seo et al. [72] used biosensors utilizing localized surface plasmon resonance (LSPR) with 2D gold nano-islands (AuNIs) and the other was a field-effect transistor (FET) based biosensor. The former targeted RdRp, ORF1ab, and E gene with a LOD of 0.22 pM while the other was against spike protein and successfully determined the virus to concentration of 100 fg/ml. Clinical observations demonstrated a clear discrimination between patient and normal samples using FET biosensor. Similarly, easy movable device bCUBE®2.0 was developed by Hyris Ltd. and proposed by Martinelli et al. [73] which can work on smartphones, tablets, laptops, and PCs with typical operating systems giving results within 2 h.

3.5. Discussion

RT-PCR being routinely used molecular detection method for various respiratory viruses makes the protocol designing for the testing of SARS-CoV-2 much easier. Hence, it was Corman et al. who initially designed and validated the assay using the virus cell cultures possessing genetic relatedness to the 2003 SARS-CoV. The development process was directed towards certain vital genes of the virus (E, RdRb, N, S and ORF1ab). Studies suggested E protein targeting was sufficient but RdRb protein targeting was recommended for confirmation. Paradoxically, analytical sensitivity of E and RdRb gene was much better compared to N gene as demonstrated by Chu et al. [29] in his study (Table 1).

Serological testing was done in a time-dependent manner owing to the sampling time and maximum studies demonstrated similar trends in the levels of IgM and IgG where the former showed positive levels in the initial stage of the infection and the latter in the later phase (Fig. 2). Comparing the sensitivity and specificity of RT-PCR and serological testing, a single method is not enough for testing SARS-CoV-2, as serological testing mainly relates to the slow response of human antibodies towards the SARS-CoV-2. Hence, it is suggested that IgM/IgG testing can be a useful diagnostic tool if combined with RT-PCR [73].

Various PoC provides advantages over the RT-PCR method such as efficiency, portability, sensitivity, and specificity. Most of the recent PoC include biosensors, LAMP/RT-LAMP based detection methods and a more recent CRISPR-Cas-12 based lateral flow assay. These PoC devices can also detect the infection at an early stage without giving any false-negative result as compared to RT-PCR which has low sensitivity for the detection of the infection at a very early stage of SARS-Cov-2 infection, but because of scarcity of investigations on clinical samples its specificity and sensitivity cannot be assured.

3.6. Limitations

Many test methods are still not evaluated clinically as the study design is poor or unclear. Therefore, studies still need to be conducted for further clinical evaluation of the newly developed diagnostic tests for assuring the sensitivity and specificity.

3.7. Conclusion

The aim of molecular and serological research is the development of detection methods that can support the clinical decision making of patients suspected with SARS-CoV-2. Results of this review indicate RT-PCR still as the gold standard with certain limitations of providing false-negative results and a laborious procedure. To overcome this, several automated systems have also been established to hasten the process where the results were consistent with the standard PCR protocol. Less samples and improper testing time because of unavailability of kits are some limitations of this study. However, none of the methods were 100% sensitive and specific; hence additional studies should be done to overcome the challenges addressed here.

Author contributions

Data conceived and retrieval was done by SM and GB. The manuscript was written by SM and GB with the final approval. PS, HK, SB and SK helped in data extraction, revision of the manuscript critically and approved the final version. For further corrections BM and AP was approached.

Declaration of competing interest

No conflict of interest.

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