Systematic Literature Review: Weaknesses and Strengths of the Latest Diagnostic Methods for COVID-19

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Abstrak

SARS-CoV-2 adalah virus RNA beruntai positif, yang merupakan Coronavirus ketujuh yang diketahui dapat menginfeksi manusia. Mengingat sifat SARS-CoV-2 yang menular dan cakupan dampaknya yang luas, mencari pengobatan yang valid adalah prioritas utama dunia. Meskipun beberapa vaksin telah digunakan, ketidakpastiannya masih belum dapat diprediksi. Mengingat hal ini, diagnosis dini SARS-CoV-2 adalah prasyarat yang efektif untuk mengekang penyebaran dan pengobatan tepat waktu, yang memungkinkan dokter melakukan intervensi tepat waktu untuk menahan penyebaran lebih lanjut dan perburukan penyakit. Penelitian ini bertujuan untuk memberikan referensi terkait kekurangan dan kelebihan deteksi Covid-19 yang berkembang saat ini. Penelitian ini merupakan Systematic Literature Review (SLR). Database yang dianalisis dalam penelitian ini berada pada rentang waktu 2019-2022 yang diperoleh dari sumber Google Scholar dengan menggunakan mesin pencarian Harzing's Publish or Perish for Windows versi 8. Berdasarkan review artikel alat uji deteksi Covid-19 dikelompokkan menjadi 3 yaitu: metode deteksi berbasis asam nukleat (RT-PCR, dPCR, mNGS, RT-LAMP, CRISPR), metode deteksi berbasis serologi (ICG/GICA, CLIA, ELISA, LFIA), dan diagnosis pencitraan CT. Masing-masing metode yang dikembangkan memiliki keunggulan dan kekurangan masing-masing. RT-PCR selalu menjadi standar utama untuk deteksi, teknik diagnostik lainnya juga berguna. Hasil gabungan mereka dapat mengecualikan hasil negatif palsu dan positif palsu sebanyak mungkin.

Kata kunci: Covid-19, Metode Diagnostik, Systematic Literature Review

Abstract

SARS-CoV-2 is a positive-stranded RNA virus, the seventh coronavirus known to infect humans. Given the infectious nature of SARS-CoV-2 and its wide-reaching effects, finding a valid treatment is a top global priority. Although several vaccines have been used, the uncertainty remains unpredictable. Considering this, early diagnosis of SARS-CoV-2 is a prerequisite for effective containment and timely treatment, allowing doctors to intervene to prevent further spread and disease worsening. This study aims to provide references regarding the advantages and disadvantages of currently developing Covid-19 detection. This research is a Systematic Literature Review (SLR). The database analyzed in this study is in the 2019-2022 timeframe obtained from Google Scholar sources using Harzing's Publish or Perish for Windows version 8 search engine. Based on an article review, the Covid-19 detection test kits are grouped into 3, namely: acid-based detection methods nucleic acid (RT-PCR, dPCR, mNGS, RT-LAMP, CRISPR), serology-based detection methods (ICG/GICA, CLIA, ELISA, LFIA), and diagnostic CT imaging. Each method developed has its advantages and disadvantages. RT-PCR has always been the gold standard for detection. Other diagnostic techniques are also useful. Their combined results can exclude false negatives and positives as much as possible.

Keywords: Covid-19, Diagnostic Method, Systematic Literature Review

1. INTRODUCTION

WHO officially announced that Covid-19 was considered a Public Health Emergency and International Concern on 31 January 2020. It indicates that the Covid-19 Pandemic can pose a risk to many countries and requires a synergistic response from all countries (Rizal et al., 2021; Wicaksono, 2020). Covid-19 is considered a pandemic that can hinder global development. Compared to SARS and MERS, COVID-19 patients also have clinical manifestations such as fever and cough accompanied by lower respiratory tract disease, especially those who are elderly and those in poor health are most susceptible to infection (Harjudin, 2020; Safriyani et al., 2021). In addition, some patients also experience

gastrointestinal symptoms, such as diarrhea, vomiting, and abdominal pain, even mild to moderate disturbances of taste and smell (WHO, 2020; Wong et al., 2020). Thrombosis is also a possible complication; even the nervous system will have signs of associated disorders.

SARS-CoV-2 is a positive stranded RNA virus, which is the seventh coronavirus known to infect humans (Fung & Liu, 2019; Kolta & Ghonimy, 2020). SARS-CoV-2 belongs to a type of coronavirus that is identical to the coronavirus that caused SARS in 2003 (SARS-CoV) and the coronavirus that caused MERS in 2012 (MERS-CoV), but Sars-Cov-2 is more contagious and there are asymptomatic patients . The genome in the formation of SARS-CoV-2 has about 80% autoploidy with SARS-CoV (Fung & Liu, 2019). The virus belongs to the Sarbecovirus subgenus of the β coronavirus (β-CoV) genus. Sequencing the viral genomes of patients with pneumonia revealed that anteriorly undiscovered β-CoV strains were present in all patients tested. The newly discovered β-CoV has 88% sequence homology with two bat-derived coronaviruses.

Given the infectious nature of SARS-CoV-2 and its wide-reaching effects, finding a valid treatment is a top global priority. The government and health workers are the main focus in dealing with the spread of this virus (Masruroh et al., 2021; Wahyono et al., 2020). Researchers have developed several drug variants by presenting various types of vaccines to prevent the spread of this virus. Although several vaccines have been used, the uncertainty remains unpredictable. Considering this, early diagnosis of SARS-CoV-2 is a prerequisite for effective containment and timely treatment, allowing doctors to intervene to prevent further spread and disease worsening. Diagnosis is a term often used in medicine. Doctors give diagnoses to patients to find out what is causing them pain. To provide a diagnosis, the doctor usually asks questions, examines the symptoms, and then identifies what makes the patient sick (Advani & Santoso, 2019; Shalihah et al., 2016). Diagnosis is a process that will help understand a person's condition and disease, likewise, in the case of COVID-19. Early detection of Covid-19 will help timely procedures for treating COVID-19. It is the key to controlling the further spread of the Pandemic. Several previous studies have shown that the diagnosis positively impacts the world of health (Ritonga & Yasthophi, 2019). This study aims to provide references regarding the advantages and disadvantages of currently developing Covid-19 detection.

2. METHODS

Methodology in literature review is divided into two groups: First, the content-based methodology focuses on an in-depth analysis of literature content to group specific knowledge according to the topic of interest. Methodologies included in this category include narrative review, critical review, SLR, mapping review, paying review, and metrics-based methodologies, which focus on a quantitative statistical analysis of literature metrics (e.g., the number of citations and the number of keywords) to understand the dynamics of a particular research area. Methodologies that fall into this category include descriptive reviews, quantitative meta-analyses, bibliometrics, and scientific metrics (Samnani et al., 2017). So this research is a Systematic Literature Review (SLR). Researchers comprehensively review the literature so that the goals set can be achieved. The SLR method guides conducting literature studies in an organized, transparent, and replicable manner (Rouhani et al., 2015). The researcher determines the Research Question (RQ) in the research design section. Our research defines two RQs: 1) What are the currently developing Covid-19 detection methods? and 2) What are the advantages and disadvantages of each method? Next is the selection of databases. The database analyzed in this study is in the 2019-2022 timeframe obtained from the source https://scholar.google.co.id/ using Harzing's Publish or Perish for Windows version 8 search engine. Entering the keywords used: detection covid-19; RT-PCR; digital-

PCR; mNGS; RT-Lamp; CRISPR-based assays; GICA; CLIA; ELISA, LFIA, CT imaging. The engine displays 200 searches based on the keywords entered. The search list that appears is selected according to the RQ, while unrelated ones are ignored. The final activity is to combine (Synthesis) all the articles that have been determined to be analyzed based on the RQ that has been determined at the beginning

3. RESULTS AND DISCUSSION

Result

Search results using the Publish or Perish application are limited to 200 lists, and then the manual selection is carried out to obtain articles that support the research objectives. Details of the selection of search results are presented in Table 1.

Table 1. Details of Search Selection

No	Selection Details	Total
1	Invalid (not an article)	92
2	Inappropriate title	40
3	The title is appropriate, but the content is not	38
4	Not quantitative research	20
5	Selected articles	10
	Total	**200**

The selection results of the 30 selected articles were analyzed based on RQ 1 and RQ 2 so that the results are as shown in Table 2.

Table 2. Analysis of selected articles

No	Researcher	Title	Covid-19 Detection Method	Result
1	(Mei et al., 2020)	Clinical features of patients infected with the 2019 novel coronavirus in Wuhan, China	RT-PCR	Real-Time Polymerase Chain Reaction is a molecular biology method by converting Sars-Cov-2 RNA into complementary DNA
2	(Falzone et al., 2020)	Sensitivity assessment of digital droplet PCR for SARS-CoV-2 detection	dPCR	Digital Polymerase Chains Reaction is a refinement of RT-PCR, which is used to measure nucleic acid strands directly
3	(Sauter et al., 2020)	Insights into the pathogenesis of fatal COVID-19 pneumonia from histopathology with immunohistochemical and viral RNA studies	mNGS	Metagenomics next-generation sequencing (mNGS) is a high-throughput detection method to obtain viral genome information in a short time
4	(Gui & Jiang, 2014)	Development and Validation of a Rapid, Single-Step Reverse Transcriptase Loop-Mediated Isothermal Amplification (RT-LAMP) System Potentially to Be Used for Reliable and High-Throughput Screening of COVID-19	RT-LAMP	Reverse-transcription loop-mediated isothermal amplification is a one-step nucleic acid amplification method that combines loop-mediated isothermal amplification (LAMP) and reverses transcription

No	Researcher	Title	Covid-19 Detection Method	Result
5	(Ding, K. Yin, Z. Li, R.V. Lalla, E. Ballesteros, M.M. Sfeir, 2020)	Ultrasensitive and visual detection of SARS-CoV-2 using all-in-one dual CRISPR-Cas12a assay	CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-related proteins (CRISPR/Cas) are part of the acquired immune system.
6	(Mei et al., 2020)	A facile assay for rapid detection of COVID-19 antibodies	ICG	The immunochromatographic assay is a method of coating the SARS-CoV-2 antigen on a nitrocellulose membrane and capturing SARS CoV-2 IgM/IgG antibodies in human serum based on the principle of lateral immunochromatography.
7	Rai *et al.* (2021)	Detection technologies and recent developments in the diagnosis of COVID-19 infection	CLIA	CLIA is a highly sensitive immunoassay that combines the technology of a highly sensitive chemiluminescence assay with a highly specific immune response.
8	(Xu et al., 2020)	Unique epidemiological and clinical features of the emerging 2019 novel coronavirus pneumonia (COVID-19) implicate special control measures	ELISA	The ELISA method to fix the SARS-CoV-2 antigen to the carrier, which is used to capture the SARS-CoV-2 IgM/IgG antibodies
9	(Kontou et al., 2020)	Antibody Tests in Detecting SARS-CoV-2 Infection	LFIA	The principle of LFIA detection is: that the antigen is placed on the detection strip. The test sample and labeled antibody move along the strip. If the SARA-CoV-2 antibody is present in the sample, it will combine with the labeled antibody and antigen to form a complex, and then a band will appear
10	(Mei et al., 2020)	Artificial intelligence-enabled rapid diagnosis of patients with COVID-19	CT	CT imaging-assisted diagnosis is an additional method of diagnosis by analyzing CT images of patients who have been diagnosed

Discussion

Real-Time Polymerase Chains Reaction (**RT-PCR**)

Following WHO requirements, the gold standard for detecting COVID-19 is RT-PCR. The PCR tool has high sensitivity and specificity and has always been a routine method for detecting the coronavirus (Drame et al., 2020). Generally, sampling the irritating upper respiratory tract as the test substance (Takeuchi *et al.*, 2020), such as phlegm and nasal discharge, is the best test. The detection method using specific primers and probes for the SARS-CoV-2 nucleocapsid gene has the highest analytical sensitivity. For patients diagnosed with COVID-19, the results of the RT-PCR test can be used as a standard for isolation and discharge. The principle of detection is: The RNA in the SARS-CoV-2 genome is converted

into complementary DNA (cDNA). DNA polymerase modifies DNA via a cDNA template (Chen et al., 2020). RT-PCR reactions were monitored using fluorescent dyes or TaqMan DNA probes. RT-PCR detection steps include sample collection, RNA extraction, polymerase chain reaction, and real-time analysis. PT-PCR Covid-19 detection scheme is presented in Figure 1.

RT-PCR method with respiratory tract, blood, and feces samples from infected people is detected (Huang et al., 2020). The detection results showed that patients aged 25-49 accounted for the highest proportion of 49%, and most were male. The PCR polymerase chain reaction can directly measure the number of nucleic acid molecules in the initial sample, calculate the absolute value of the nucleic acid concentration, and is a new age-making nucleic acid detection technology. However, RT-PCR test results cannot completely exclude false negatives. The mismatch between the primer, probe, target sequence, and sampling procedure will decrease detection performance and false negative results (Lan et al., 2020).
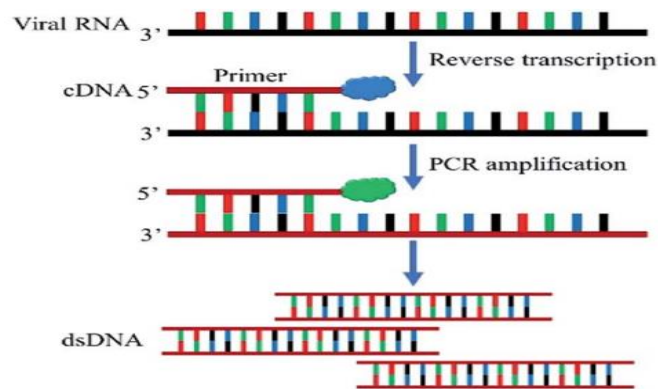

Figure 1. RT-PCR Covid-19 detection scheme

Digital polymerase chain reaction (dPCR)

Digital PCR can directly measure the number of nucleic acid molecules in the initial sample, calculate the absolute value of the nucleic acid concentration, and is a new age-making nucleic acid detection technology (Alteri et al., 2020). Compared with RT-PCR, the main advantages of dPCR are reflected in high detection accuracy, high sensitivity, stable system, and excellent repeatability (Falzone et al., 2020). dPCR divides the nucleic acid molecule into several reaction units, and each unit performs independent PCR amplification (Xu et al., 2020). Reaction principle is shown in Figure 2.
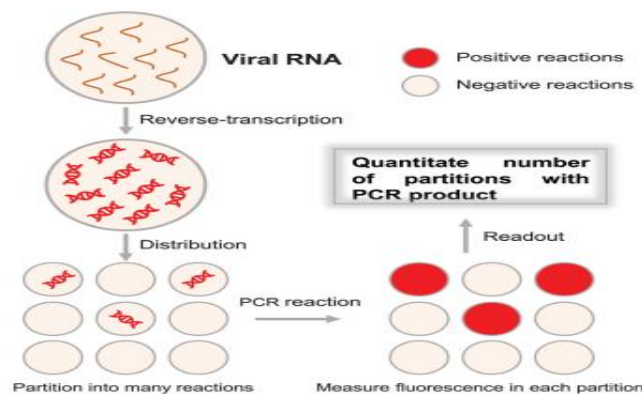

Figure 2. Schematic of dPCR detection

Digital dPCR shows higher sensitivity than RT-PCR. False-negative and false-positive dPCR test results are much less than RT-PCR, especially for trace sample detection. However, the disadvantages of digital PCR are also obvious, such as high technical cost, complicated operation, limited detection throughput, and difficulty in popularizing and applying it in epidemic areas and primary medical units.

Metagenomics next-generation sequencing (mNGS)

Genetic sequencing of the pathogen is the most accurate identification method for emerging infectious diseases. In the study of the COVID-19 genome, scientists have used the platform's high-throughput detection to obtain first-hand information about the viral genome in a very short time (Gu et al., 2020). The basic process is to extract viral RNA from the patient's lower respiratory tract secretions, build a genetic database in the formation of the virus, then perform high-throughput sequencing, and identify whether the genome sequence is homologous to that of SARS-CoV-2 through database comparison analysis. Extraction of SARS-CoV-2 viral RNA from autopsy sections, performing NGS to detect the virus, and incorporating immunohistochemistry to explore pathogenic mechanisms (Sauter et al., 2020). Through mNGS technology, it was found that COVID-19 patients in many European countries had an amino acid deletion of nsp2 (Asp268Del). However, mNGS detection technology also has limitations: such as short read lengths, uneven genome coverage, susceptibility to contamination of the host genome, and high cost. Therefore, it is too early to use high-throughput sequencing as a census tool at this stage, but it is critical for sequencing and genetic analysis of new respiratory pathogens.

Reverse-transcription loop-mediated isothermal amplification (RT-LAMP)

RT-LAMP is a one-step nucleic acid amplification method that combines loop-mediated isothermal amplification (LAMP) and reverses transcription. The detection principle of RT-LAMP is shown in Figure 3.
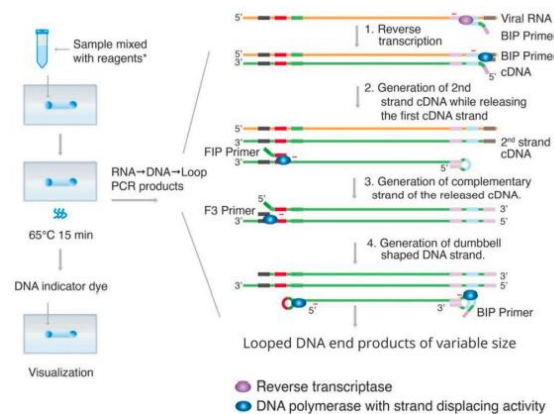

Figure 3. PT-LAMP detection scheme

The new method is similar to traditional polymerase chain reaction detection, except the nucleic acid amplification is carried out at the same temperature. Compared with RT-PCR, RT-LAMP has clear advantages. For example, certain equipment for polymerase chain reaction is no longer required and has the advantages of easy operation, simple use, speed, high sensitivity, and specificity. It enables fast screening, greatly shortening the detection time. However, due to the high sensitivity of RT-LAMP, it is very susceptible to contamination and produces false positive results, so contamination of operations must be strictly prevented (Verma et al., 2020).

CRISPR-based assays

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) first generate RNA sequences complementary to the target sequence and can be targeted for binding to viral or plasmid DNA. Cas ribonucleic acid was used for double-strand cleavage targeting complementary sequences. The CRISPR-based test detection scheme is presented in Figure 4. Studies in recent years have shown that it can be used to detect viral nucleic acids with high sensitivity, simplicity, and reliability of results. A multifunctional detection method, dual-CRISPR-Cas12a (AIOD-CRISPR) with high sensitivity (Ding, K. Yin, Z. Li, R.V. Lalla, E. Ballesteros, M.M. Sfeir, 2020). By targeting the gene of the nuclear protein SARS-COV-2, two CRISPR RNAs without protospacer adjacent motif site restriction (PAM) were introduced, thus forming the AIOD-CRISPR detection method. This analysis was validated using COVID-19 clinical swab samples, and the results agree with reverse transcription-polymerase chain reaction analysis.
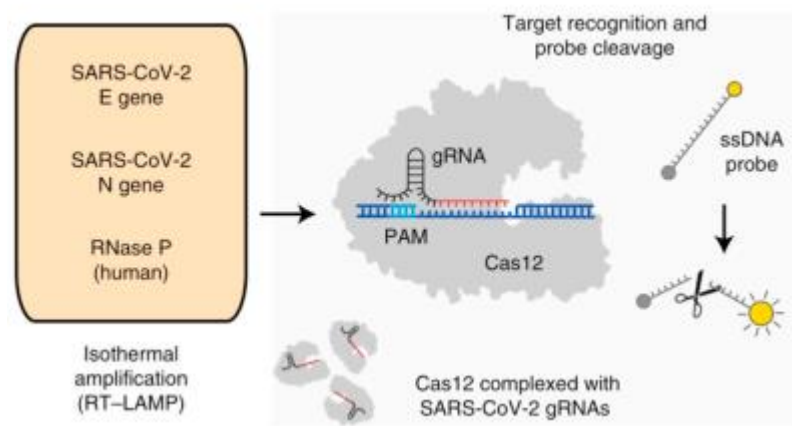

Figure 4. CRISPR-based test detection scheme

Colloidal gold immunochromatographic assay (GICA)

The colloid gold method coats the SARS-CoV-2 antigen on a nitrocellulose membrane and captures SARS CoV-2 IgM/IgG antibodies in human serum based on the principle of lateral immunochromic matography. The sample detected by the colloid gold method comes from the patient's fingertip or venous blood. The results can be observed visually only after 10-15 minutes of the immune response. The GICA detection principle is shown in Figure 5.
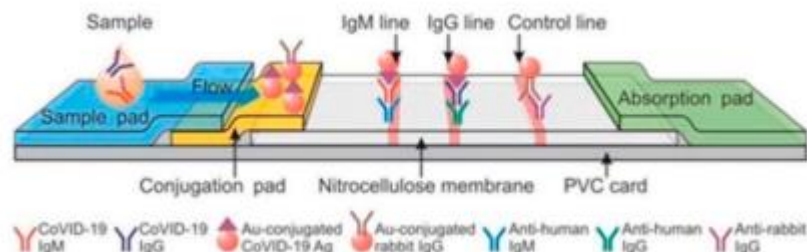

Figure 5. GICA-based test detection scheme

Colloidal gold nanoparticles have the advantages of strong stability, easy-to-read detection results and simple operation compared to other reporter markers. Also, colloidal gold nanoparticles have very low biological toxicity and good biocompatibility. Therefore, serological immunochromatography technology based on colloidal gold nanoparticles is very

effective for rapid on-site detection. The test kit based on GICA technology takes 10-15 minutes to get the test results (Mei et al., 2020). The kit has no special requirements for equipment and personnel and shows great potential for rapid, large-scale screening. Although it cannot completely replace RT-PCR technology, it can be an important additional technology. However, the IgM/IgG antibody colloid gold method as serological evidence cannot replace RT-PCR status as pathogenic evidence Test results can be affected by factors such as hemolyzed samples, fibrin, or patient autoantibodies, contributing to high false positive rates.

Chemiluminescence enzyme immunoassay (CLIA)

CLIA is a highly sensitive immunoassay that combines the technology of a highly sensitive chemiluminescence assay with a highly specific immune response. CLIA is used to detect SARA-CoV-2 antibodies in COVID-19 patients. High-precision results can be achieved when the antibody concentration reaches a critical value. The fully automated CLIA performance and the results show that its specificity and sensitivity reach a high level. The combination of CLIA technology and nucleic acid detection can greatly improve detection accuracy. Magnetic chemiluminescence enzyme antibody immunoassay is based on chemiluminescence detection, adding magnetic nanoparticles, so that the detection has higher sensitivity and faster detection speed. However, the magnetic particle chemiluminescence method needs better selectivity and will respond to a range of non-specific compounds. Therefore, the accuracy could be better. In addition, the environment has a relatively large influence on detection, which is easy to cause errors.

Enzyme-linked immunosorbent assay (ELISA)

The ELISA method to fix the SARS-CoV-2 antigen to the carrier, which is used to capture the SARS-CoV-2 IgM/IgG antibodies. Enzyme-labeled anti-IgM/IgG antibodies are used as secondary diagnostic antibodies to build an indirect detection system for IgM/IgG antibodies and quantitatively detect IgM/IgG antibodies in samples via an enzymatic color reaction. The detection sensitivity and specificity of the ELISA method are high, the operation method is simple, and the equipment configuration requirements are low. However, manual operation of the ELISA method can lead to unnecessary operational errors and cross-contamination. There is a window period for IgM/IgG antibody detection, making it difficult to obtain a positive test result in the early stages of infection (Xu et al., 2020). Compared to the colloid gold method, the advantage is that it can quantitatively detect IgM/IgG antibodies, assess the dynamic process of IgM antibodies becoming negative, increase IgG antibodies, and help monitor changes in the patient's condition.

Lateral flow immunochromatographic assay (LFIA)

The principle of LFIA detection is: that the antigen is placed on the detection strip. The test sample and labeled antibody move along the strip. If the SARA-CoV-2 antibody is present in the sample, it will combine with the labeled antibody and antigen to form a complex, and then a band will appear, as shown in Figure 6 (Yoshimasa Takeuchi et al., 2021). This detection technology is inexpensive, simple to operate, and does not require suitable instruments. However, the detection sensitivity cannot reach the CLIA and ELISA levels, and the detection results must be carefully considered (Kontou et al., 2020).
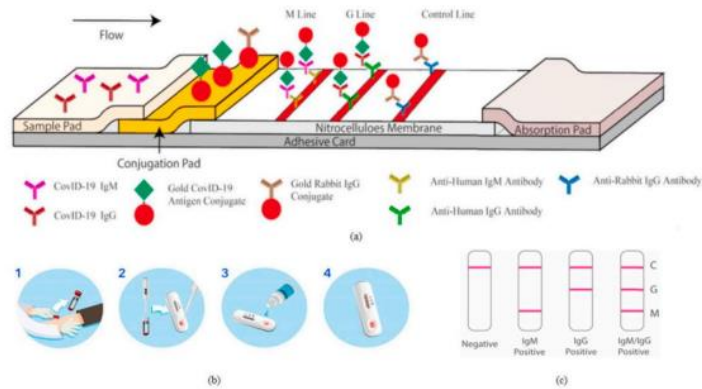
Figure 6. LFIA-based test detection scheme

CT imaging-assisted diagnosis

CT imaging is a good adjunctive method of diagnosis using CT images to aid in the analysis of COVID-19. CT images of pathologically confirmed, representative viral pneumonia cases previously diagnosed. The algorithm framework is shown in Figure 7.
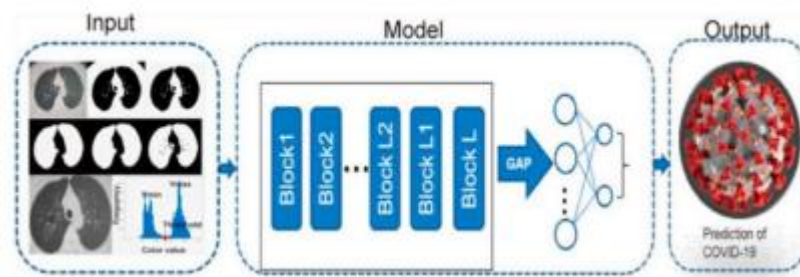

Figure 7. CT imaging-assisted diagnosis algorithm framework

This system includes three main processes: 1) Image pre-processing. 2) Feature extraction and ROI image training. 3) Classification and prediction. Artificial intelligence algorithm with CT display, clinical symptoms, and laboratory tests to quickly diagnose COVID-19 patients (Mei et al., 2020). Diagnosis with the aid of CT imaging as the mainstay of acid detection and complementary serum detection, the sensitivity and accuracy of detection should be improved as much as possible. With the development of global cooperation, there will be greater and faster breakthroughs in diagnosing COVID-19. The first part is a chest CT scan. The next section is classifying with clinical information. The last part is to produce classification results. Although chest CT results may not be as accurate as nucleic acid detection, they can be used as a valuable tool and reference. Given the lack of nucleic acid detection kits and long detection times, the advantages of intelligent detection systems are obvious.

Discussions

SARS-CoV-2 is a positive stranded RNA virus, which is the seventh coronavirus known to infect humans (Fung & Liu, 2019; Kolta & Ghonimy, 2020). SARS-CoV-2 belongs to a type of coronavirus that is identical to the coronavirus that caused SARS in 2003 (SARS-CoV) and the coronavirus that caused MERS in 2012 (MERS-CoV), but Sars-Cov-2 is more contagious and there are asymptomatic patients . The genome in the formation of SARS-CoV-2 has about 80% autoploidy with SARS-CoV (Fung & Liu, 2019). The virus belongs to

the Sarbecovirus subgenus of the β coronavirus (β-CoV) genus. Sequencing the viral genomes of patients with pneumonia revealed that anteriorly undiscovered β-CoV strains were present in all patients tested. The newly discovered β-CoV has 88% sequence homology with two bat-derived coronaviruses.

Given the infectious nature of SARS-CoV-2 and its wide-reaching effects, finding a valid treatment is a top global priority. The government and health workers are the main focus in dealing with the spread of this virus (Masruroh et al., 2021; Wahyono et al., 2020). Researchers have developed several drug variants by presenting various types of vaccines to prevent the spread of this virus. Although several vaccines have been used, the uncertainty remains unpredictable. Considering this, early diagnosis of SARS-CoV-2 is a prerequisite for effective containment and timely treatment, allowing doctors to intervene to prevent further spread and disease worsening. Diagnosis is a term often used in medicine. Doctors give diagnoses to patients to find out what is causing them pain. To provide a diagnosis, the doctor usually asks questions, examines the symptoms, and then identifies what makes the patient sick (Advani & Santoso, 2019; Shalihah et al., 2016). Diagnosis is a process that will help understand a person's condition and disease, likewise, in the case of COVID-19. Early detection of Covid-19 will help timely procedures for treating COVID-19. It is the key to controlling the further spread of the Pandemic. Several previous studies have shown that the diagnosis positively impacts the world of health (Ritonga & Yasthophi, 2019).

4. CONCLUSION

Since the outbreak of COVID-19, various test kits have appeared one after another. Based on the article review, the Covid-19 detection test kits are grouped into 3: nucleic acid-based detection methods (RT-PCR, dPCR, mNGS, RT-LAMP, CRISPR), serology-based detection methods (ICG/GICA, CLIA, ELISA, LFIA), and CT imaging diagnosis. Each method developed has its advantages and disadvantages. RT-PCR has always been the gold standard for detection. Other diagnostic techniques are also useful. Their combined results can exclude false negatives and positives as much as possible.

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