Am J Transl Res 2020;12(10):6763-6774
www.ajtr.org /ISSN:1943-8141/AJTR0118609

Review Article

Progresses in nucleic acid testing for COVID-19

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Received March 23, 2020; Accepted October 1, 2020; Epub October 15, 2020; Published October 30, 2020

Abstract: COVID-19 (Coronavirus disease 2019) epidemic has rapidly spread since its outbreak. By 24:00, July 19, China had reported 83,682 confirmed infectious cases of COVID-19, including 4,634 deaths. The prevention and control of COVID-19 remains extremely urgent. Owing to its strong infectivity and onset in populations, early detection of infectious cases of COVID-19 is of great significance to control the epidemic. Nevertheless, clinical experiences in nucleic acid testing (NAT) are limited. False negative results of NAT inconsistent with clinical diagnosis are often reported. Therefore, it is necessary to improve the sensitivity and specificity of NAT. This study aims to summarize the current situation and prospect of NAT application based on the lasted findings on COVID-19 infection. Meanwhile, potential methods are proposed to improve the validity of NAT, like improving sample quality. The review may provide references for clinical and experimental explorations on COVID-19.

Keywords: COVID-19, pathogenesis, quality control, results interpretation, NAT

Introduction

In China, COVID-19 (Coronavirus disease 2019) epidemic emerged in Wuhan in December 2019. In January 30th, 2020, the WHO (World Health Organization) announced the ongoing COVID-19 pandemic as a Public Health Emergency of International Concern, which is the sixth after the SARS (Severe Acute Respiratory Syndrome) epidemic. It is clarified that the receptor-binding domain (RBD) within the S protein of COVID-19 may bind to angiotensin-converting enzyme 2 (ACE2) as SARS-Coronavirus (SARS-CoV) RBD does [1]. The clinical symptoms of COVID-19 include fever, dry cough, malaise and dyspnea. During the early phase of COVID-19 infection, most of patients have normal or decreased white blood cell counts, decreased lymphocyte counts, increased C-reactive protein level, normal platelet level and imaging findings. For severe COVID-19 cases, they have significantly increased levels of pro-inflammatory cytokines (e.g. IL-6, TNF-α and INF-γ), displaying the characteristic of cytokine storm [2]. The first autopsy of COVID-19 case was performed by Xu et al. [3]. They reported the pulmonary pathological findings, including diffuse alveolar injury and hyaline membrane formation, similar to those of acute respiratory distress syndromes, like SARS and MERS (Middle East Respiratory Syndrome). Since the first infectious case of COVID-19 in Wuhan, the epidemic has rapidly spread to other cities in China and 26 foreign countries [3]. By 24:00, July 19, China had reported 83,682 confirmed infectious cases of COVID-19, including 4,634 deaths [4]. Currently, special treatment against COVID-19 infection is lacked. Diagnosis and management of COVID-19 as early as possible are very critical to control the spread. Outlines of 2019 Novel Coronavirus Diagnosis and Treatment issued by the National Health Commission of the People's Republic of China suggested that nucleic acid testing (NAT) is a diagnostic standard for COVID-19 infection [5]. So far, eight NAT kits have been approved by

the National Medical Products Administration for diagnose of COVID-19 infection.

Owing to the seriousness of COVID-19 epidemic, many newly developed kits are immediately applied in clinical practice. The insufficient verification of kits performance results in false negative cases that are inconsistent with clinical diagnosis. For example, a COVID-19 patient admitted in China-Japan Friendship Hospital had three negative NATs of throat swabs. At a hospital in Hangzhou, a COVID-19 case was finally confirmed by the seventh NAT. Thus, clinical significance of NAT for diagnosing COVID-19 infection has been largely doubted [6]. This study aims to summarize the current situation and prospect of NAT application based on the lasted findings on COVID-19 infection. Meanwhile, potential methods are proposed to improve the validity of NAT, like improving sample quality.

Etiology, transmission and pathogenesis of COVID-19

By performing the metagenomics next generation sequencing (mNGS) in bronchoalveolar lavage fluid samples, COVID-19 was isolated [7]. Based on phylogeny, taxonomy and established practice, the International Committee on Taxonomy of Viruses (ICTV) officially recognized this virus as a sister to SARS-CoV, and designated it as SARS-CoV-2 [8]. Later, the WHO referred the pneumonia caused by SARS-CoV-2 as COVID-19. Structurally, COVID-19 belongs to the family of coronaviruses [9], with a highly genetic similarity to bat coronaviruses (over 85% identical) [10]. COVID-19 shares 78% and 50% genetic sequence with SARS and MERS, respectively [11]. It is generally considered that Rhinolophus sinicus is the natural reservoir of COVID-19. A latest research proposed that pangolins may be the intermediate host (85.5%-92.4% identical) involved in the introduction of COVID-19 to humans [12].

A novel finding suggested that COVID-19 may spread through aerosol transmission under certain circumstance. Very recently, detection of COVID-19 in stool samples indicated the possibility of fecal-oral transmission [13]. So far, no evidences have supported COVID-19 infection caused by contaminated food, but the possibility of aerosol or contact transmission after fecal excretion cannot be excluded [11]. The urinary system may also be a potential transmission route for COVID-19 infection [14]. Jin et al. demonstrated that ACE2, the SARS-CoV-2 receptor, is lowly expressed in various cell types of the human maternal-fetal interface [15]. It is indicated that there may be no potential susceptible cell subpopulation of SARS-CoV-2 in the maternal-fetal interface, which attributed to the conclusion that SARS-CoV-2 infection does not cause vertical transmission of mother and fetus. In addition, experimental evidences have proven that COVID-19 cannot be transmitted through skin contact.

The pathogenesis of COVID-19 remains largely unclear. It was reported that the entry of SARS-CoV-2 into host cells requires the binding between the S protein and ACE2 on the cell membrane [16]. During the processes of viral replication, amplification and release, the body defense response is initiated. SARS-CoV-2 infection is the trigger for COVID-19. The severity of COVID-19 and viral loads are closely related to body immune function. Most of COVID-19 infected patients can be cured by adjuvant therapy via clearing viruses and repairing inflammatory damage through autoimmune function. In addition, over-activated inflammatory response and cytokine storm are responsible for the pathogenesis of viral pneumonia. ACE2 is the binding receptor of SARS-CoV-2. A single N501T mutation (corresponding to S487T mutation in SARS-CoV-2) may significantly enhance the binding affinity between COVID-19 RBD and human ACE2 [16]. ACE2, extensively expressed in human tissues, is abundant in alveolar epithelium, intestinal epithelium and vascular endothelial cells. Pathological lesions in lung are the major characteristics of COVID-19, and diarrhea is uncommon, suggesting that lung is the target organ of COVID-19 [17]. The release of cytokines (e.g. MCP-1, GM-CSF, M-CSF) is induced by viral infection, and they are activated after binding corresponding receptors on the surface of macrophages. The activated macrophages not only initiate the specific immune response by recruiting abundant mononuclear phagocytes, but also induce tissue damages by releasing inflammatory factors (e.g. IL-1β, TNF-α, IL-6, MCP-1). MCP-1 could stimulate the synthesis of angiotensin II (Ang II), further aggravating the inflammatory response [18]. The latest research has proven the involvement of inflam-

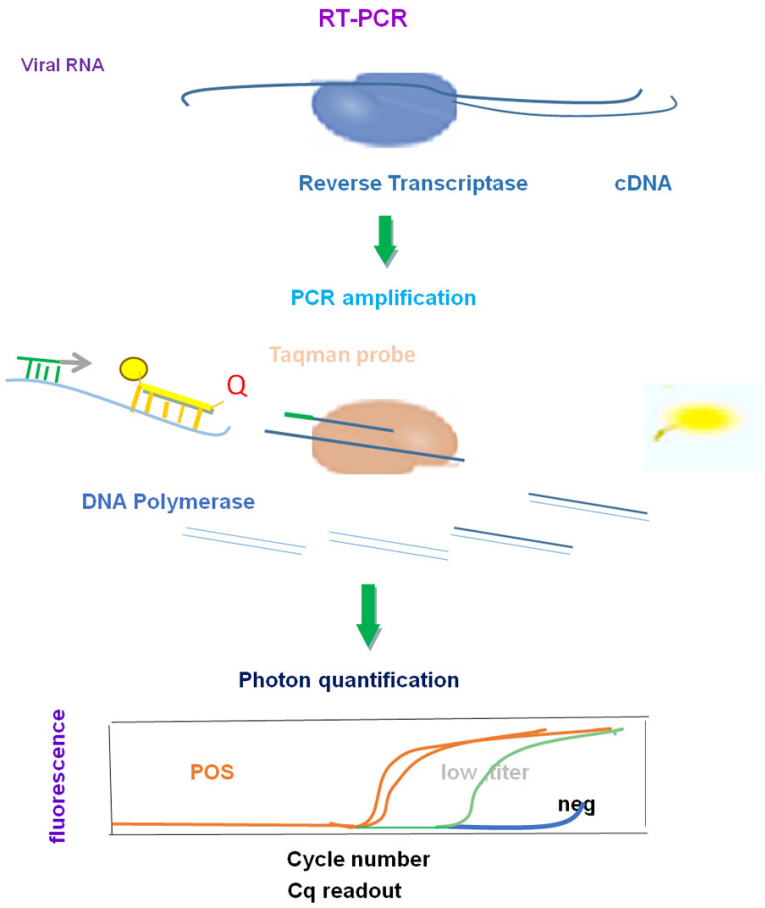

Figure 1. Meagan N Esbin, Oscar N Whitney, Shasha Chong, Anna Maurer, Xavier Darzacq, Xavier Darzacq, Robert Tjian. Overcoming the bottleneck to widespread testing: a rapid review of nucleic acid testing approaches for COVID-19 detection [21]. Steps of a new assay of coronavirus nucleic acid (RT-PCR reaction). Taqman probes are used to visualize increased fluorescence during each cycle of amplification. Amplification is quantified by C_q readout and a threshold is set for positive detection of the target amplicon.

matory cascade in pulmonary lesions, such as alveolar edema or inflammatory exudation [19]. Nevertheless, potential mechanisms underlying the development of inflammatory cascade following SARS-CoV-2 infection are unclear. It is speculated that SARS-CoV-2 infection induces the release of TNF-α, IL-1, interferons and chemokines by activating immune cells. A large number of immune cells are aggregated and infiltrated in lung. Meanwhile, intracellular inflammation-related pathways are activated. The inflammation cascade is initiated and further stimulates the release of abundant cytokines to activate more inflammatory cells. Such a vicious circle finally leads to cytokine storm. Serving as the sensitive cell receptor of SARS-CoV-2, ACE2 also exerts a critical role during the process of inflammatory response. In ACE/Ang II-induced lung injury model, ICAM-1 is up-regulated following the activation of NF-κB, resulting in the increase in vascular permeability and aggregation of pulmonary edema. The binding between SARS-CoV and ACE2 declines the activity of ACE2 and inhibits the generation of angiotensin-(1-7), whereas systemic level of Ang II from Ang I increases. The accumulation of excessive Ang II further aggravates the inflammatory response [20].

NATs for COVID-19 infection

Detection principles and results interpretation of currently applied COVID-19 NATs vary a lot, each having its advantages. However, some of testing methods do not show sufficient sensitivities. Multiple laboratory technologies, including digital polymerase chain reaction (dPCR), mNGS, gene editing technology, isothermal amplification and nucleic acid massspectrometry, are conductive to enhance the detective rate of COVID-19.

RT-PCR

Detection principles: RT-PCR is the major method for clinical diagnosis of COVID-19 (**Figure 1**) [21]. PCR products are labeled and tracked using the fluorescence-labeled specific probe for timely observing their amplification. The amount of initial template is calculated based on the amplification curves. The COVID-19 genome is arranged in the order of 5'-replicase (open reading frame 1ab, ORF1ab)-structural proteins [Spike (S)-Envelope (E)-Membrane (M)-Nucleocapsid (N)]-3' [22]. By comparing sequence with other coronaviruses, it is found that ORF1ab and Nucleocapsid are specific for coronavirus. Therefore, specific primers targeting ORF1ab and Nucleocapsid can be used for detecting COVID-19. Primers targeting Spike are also used in some reagents aiming to enhance the detective sensitivity. Compared

with gene sequencing technologies, RT-PCR detection of COVID-19 has advantages of reduced assay time, simple procedures, and pronounced performances in specificity and repeatability [23]. Chu et al. suggested that detecting Nucleocapsid of COVID-19 genome has a better sensitivity, as compared with ORF1ab detecting [19]. Corman et al. established and optimized the RT-PCR procedures for COVID-19 detection [24]. COVID-19 cases are confirmed by both probes positive for one sample, or one probe positive for two samples, or one probe positive for one sample twice. Research institutes and companies have made great efforts on developing NAT kits as fast as they can, including one-step and two-step RT-PCR reagents. Robust diagnostic methodology of NAT should take consideration into actual situations. In addition, the approval of novel NAT kits for COVID-19 detection has been significantly accelerated by the National Medical Products Administration. In January 26th, 2020, four COVID-19 NAT kits were emergently approved, and another four have been approved in succession thereafter. It is conductive to rapid diagnosis and active management of COVID-19 infection (Such analysis is only for China's policies and products).

Quality control

The approved kits lack sufficient verification, leading to contradictory results. Li et al. detected 255 samples collected from COVID-19 cases using two NAT kits [25]. Only 77.25% of the cases received same results. Besides, they proposed that different components solutions in sampling tubes may result in false negative results. Thus, quality control during all NAT procedures should be performed.

Sample collection

The quality of collected samples is the determinant factor for the efficiency of NAT. The lower respiratory tract is the major part attacked by COVID-19 infection. Theoretically, sputum from deep respiratory tract or bronchoalveolar lavage fluid (BALF) is the ideal sample for COVID-19 detection. However, to prevent nosocomial infection, only nasopharynx or oropharynx swabs are collected in clinical practice. Nylon flocked swabs with fine brushes on the surface are recommended. Surface mucosal cells are collected by swabbing the posterior pharyngeal wall, crypt and lateral wall of tonsil beyond the root of tongue for 3-5 times. Meanwhile, multisite samples from the same case are recommended to be collected for improving the detection efficiency (e.g. oral and bilateral nasopharynx swabs).

Potential influences of inflammatory cytokine storm on NAT efficiency

After 2019-nCoV infection, the immune cells may be over-activated and produce a large number of inflammatory factors, forming an "inflammatory cytokine storm" through the positive feedback regulation mechanism. Inflammatory exudation in lung of COVID-19 cases may be attributed to the inflammatory cytokine storm [26, 27]. The inflammatory cytokine storm is a double-edged sword, and while an over-activated immune response can clear the virus quickly, it can also cause temporary false negatives of NAT. However, excessive activation of the immune response can also damage normal cells, and severe disease conditions can be observed in clinical practice. Immune-induced pathogenesis is identified in the cases of viral hepatitis as well. Clinical diagnosis is required for negative NAT cases with typical pulmonary symptoms in areas where COVID-19 is endemic, which significantly avoids missed diagnosis of severe patients. For suspected severe patients, sputum of deep respiratory tract or BALF samples should be examined.

Time of inspection

The positive rate of NAT is relatively higher in the fastigium of virus replication. Under the situation of limited reagents and manpower, it is necessary to perform NAT for COVID-19 patients in their acute phase. Samples (multisite samples) suspected of COVID-19 infection are necessary to be retained according to the management of infectious diseases under Class A [28].

Potential influences of differences in sample processing and NAT kit protocols on RNA extraction efficacies

Manual extraction of nucleic acids in the laboratory is extremely inconvenient, owing to the high protection requirements. Therefore, automatic nucleic acid extraction system in biosafety cabinets and COVID-19 NAT kit are recommended.

In addition to using reagents approved by the National Medical Products Administration, performance verification shall be performed before the official use of the recommended reagents. In the actual test, all kinds of details should be regulated in strict accordance with the operating procedures to avoid false negative and false positive results, so as to ensure the results are fast and accurate. Performance verification parameters should include at least precision, coincidence rate and detection limit. Meanwhile, quality control and test data should be accumulated during clinical testing and compared with other laboratory results to carry out further evaluation and verification of other performance indicators (such as specificity, anti-interference ability, etc.). Through the performance verification, the optimal testing system is formed, and operable standard operating procedures are established [29].

External quality assessment for NAT by the national center for clinical laboratories

The self-developed, non-biologically risky, phage virus-like particle samples are utilized for assessing the comparability, accuracy, specificity and sensitivity of NAT in each laboratory. Testing institutions should participate in an inter-laboratory quality assessment and biosafety supervision before operating NAT, and should be qualified. In the future, they must regularly (at least once a year) participate in the inter-laboratory quality assessment and biosafety supervision organized by the municipal or other clinical testing centers at or above the provincial level, and shall be qualified. Laboratories operating NAT must establish sound standards for biosafety precautions, technical procedures, quality assurance measures, and results reporting.

Notes for results interpretation of NAT

According to the latest guideline of COVID-19 diagnosis and prevention, and consensus of laboratory experts, at least two probes targeting ORF1ab and Nucleocapsid or Envelope of COVID-19 should be used. Positive signals of both ORF1ab and Nucleocapsid or Envelope can confirm positive COVID-19. Notably, negative signals of ORF1ab and Nucleocapsid cannot exclude the possibility of COVID-19 infection, and any reasons leading to false negative results should be considered. In the COVID-19 guideline (fifth edition) issued by the National Health Commission of the People's Republic of China, it is highlighted that resampling and detection are required for the result of single positive signal of ORF1ab or Nucleocapsid. In addition, single positive signal of ORF1ab or Nucleocapsid in the second round of detection suggests the positive NAT of COVID-19. Single positive signal of ORF1ab or Nucleocapsid in two different types of samples collected from one case also suggests the positive COVID-19 infection [23].

Cases with single positive signal of ORF1ab or Nucleocapsid in NAT can be attributed to the difference in amplification sensitivity in duplex RT-PCR kits. Higher amplification sensitivity of Nucleocapsid than that of ORF1ab will lead to single positive signal in samples with relatively low viral loads. In addition, mRNA transcription of COVID-19 is similar to that of SARS in theory. The mRNA levels of COVID-19 are several times of the genomes. Transcribed mRNAs with various lengths contain Nucleocapsid, but rarely ORFab1. At present, intracellular mRNAs of COVID-19 are mainly detected using duplex RT-PCR kits, leading to higher copies of Nucleocapsid mRNAs compared with those of ORFab1. As a result, detective rate of single positive signal of Nucleocapsid is higher than that of ORF1ab in the situation of low virus copies. Notably, the nucleic acid sequence of Nucleocapsid in COVID-19 is less conserved than that of ORF1ab. Crossed nucleic acid sequences of other coronaviruses in the small number of COVID-19 cases will result in single positive signal of Nucleocapsid and negative signal of ORFab1.

Other NATs

Digital PCR: Digital PCR (dPCR) carries out a single reaction with a sample separated into a large number of nanoliter-scale partitions. Nucleic acids are randomly distributed in each partition, which contains 0, 1 or more target nucleic acids. At the end of PCR, fluorescence signals in each partition are individually calculated and analyzed. Compared with traditional PCR, dPCR is able to absolutely quantify nucleic acid amounts, independent of threshold per cycle, internal controls and standard curves [30]. At present, modified dPCR methods, including BEAMing (beads, emulsion, amplification and magnetics), ddPCR (droplet digital

PCR) and SNP-based microarrays, have been applied in methylation of tumor diagnosis, gene mutations, circulating tumor DNAs and noncoding DNAs [31]. Very recently, dPCR has been designed to measure viral loads. Oma et al. demonstrated that dPCR had higher sensitivity in detecting bovine coronavirus when compared with traditional PCR [32]. Great efforts have been made to improve dPCR methods, thus reducing the rate of false negative results for COVID-19 detection. Highly sensitive dPCR kits targeting signals of ORF1ab, Nucleocapsid and Envelope in COVID-19 were developed by the National Institute of Metrology, China. Three-channel dPCR kits for detecting COVID-19 have also been developed by commercial companies. However, they have not been approved for clinical use. dPCR is only used in a small number of high-level hospitals because of its strong dependence on the equipment and expensive cost.

Metagenomic next-generation sequencing (mNGS)

Based on the next-generation sequencing technology, mNGS identifies bacteria and viruses directly from clinically extracted DNAs and/or RNAs, followed by library preparation and bioinformatic analysis. Pre-treatment of collected samples is necessary before nucleic acid extraction, aiming to elevate the detective rate of pathogens, such as sputum liquefaction and chemical depletion for host DNA. Library preparation can be optimized via adding known and clear sequences to both ends of a nucleic acid fragment with an unknown sequence. PCR is conducted for every single library, followed by sequencing of all the nucleic acid fragments of the library. The raw data obtained from sequencing is usually cleaned, trimmed and filtered to remove human host and low-quality sequences. Finally, the comprehensive analysis and interpretation are obtained based on the preliminary results of the automation system, and some clinical indicators (e.g. sample types, pathogen types). In 2003, the identification of SARS took more than 5 months. By comparison, genome identification and analysis of COVID-19 was completed within as short as 5 days. Ren et al. reported a descriptive study to illustrate the workflow of COVID-19 identification [33]. They collected clinical data and BALF samples from five patients with severe pneumonia in a hospital of Wuhan, Hubei province, China. mNGS results revealed the presence of a previously unknown β-CoV strain. It had 79% nucleotide identity with the sequence of SARS-CoV and phylogenetically closest to a bat SARS-like CoV. Combined with morphological observation and serological test on the isolated virus, a novel CoV was identified. During the same period, Chen et al. extracted total RNAs from BALF samples in two patients with acute respiratory syndromes and subjected to mNGS [34]. After sequencing splicing, homology and SNP analyses, the sole pathogen in two samples with very high viral load was detected. Although some SNP profiles were identified from the mNGS data, the genome sequences obtained from the two patients were identical, indicating that two individual patients had been infected by the same CoV at different time points. The same conclusion is also yielded by Zhu et al. after genome identification in 4 BALF samples using the Illumina and nanopore platforms [35]. Currently, the genome sequence of COVID-19 is available on GISAID and Genbank. A high identity between the published sequence of COVID-19 and that obtained from the patient's genome sequencing results is the diagnostic basis for COVID-19 infection. RT-PCR is methodologically different from mNGS. Existing RT-PCR reagents mainly target signals of ORF1ab and Nucleocapsid in COVID-19. Besides signals of ORF1ab and Nucleocapsid, mNGS targets other regions in COVID-19. It not only identifies mutations outside the regions that RT-PCR detects, but also obtains more sequence information to assemble genome sequences, traces evolution resource, and predicts virus transmission. Meanwhile, mNGS provides basis for the accurate diagnosis of other pathogens and mixed infection. The use of mNGS, however, requires complicated procedures and long time. As a result, mNGS is unlikely to replace the traditional RT-PCR for the large-scale and quick diagnosis of COVID-19. Combined application of mNGS and RT-PCR is recommended.

Isothermal amplification

Isothermal amplification is extensively applied in rapid pathogen detection and molecular diagnosis owing to its low equipment dependence, effective amplification of nucleic acids, high sensitivity and specificity. According to

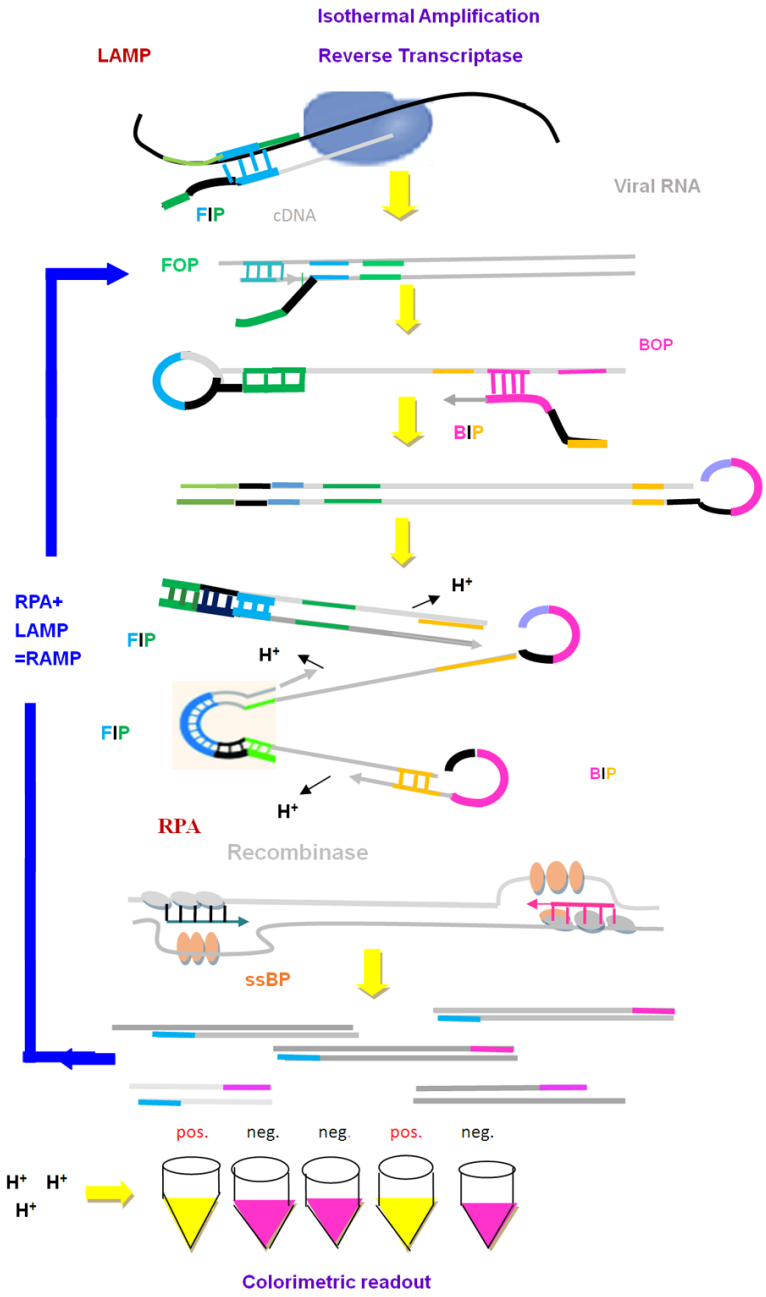

Figure 2. Meagan N Esbin, Oscar N Whitney, Shasha Chong, Anna Maurer, Xavier Darzacq, Robert Tjian. Overcoming the bottleneck to widespread testing: a rapid review of nucleic acid testing approaches for COVID-19 detection [21]. Steps of a new assay of coronavirus nucleic acid (LAMP). Molecular overview of isothermal amplification techniques. LAMP uses specially designed nested primers with complementary regions that form hairpins to permit priming of subsequent rounds of amplification. RPA uses recombinase-catalyzed strand invasion to prime amplification. Colorimetric pH indicators can be used to detect hydrogen ion release during dNTP incorporation.

reaction principles, isothermal amplification could be classified into loop-mediated isothermal amplification (LAMP), recombinase polymerase amplification (RPA), nucleic acid sequence-based amplification (NASBA), strand displacement amplification (SDA) and rolling circle amplification (RCA). Some of these have been commercially used [36]. LAMP is the most used isothermal amplification established by Notomi et al. in 2000 [37]. At a constant temperature, LAMP is carried out to achieve rapid amplification of nucleic acids using a single enzyme (**Figure 2**) [21]. Detection of strand displacement activity, primers and endpoint are the key events during LAMP. Unlike traditional DNA polymerase, the strand-displacing DNA polymerase in LAMP can catalyze primer extension using one strand of double-stranded DNA as the template. Eventually, a newly synthesized complementary strand replaces the other strand. LAMP allows easy visualization of amplification products using intercalating dyes. Combined with CRISPR, LAMP has been progressively improved in rapid and accurate detection of RNAs. Successful detection of pathogens using LAMP has been reported. ORF and Nucleocapsid proteins in MERS-CoV could be precisely identified by LAMP, and its detective efficacy is prior to the traditional RT-PCR [38]. Since the outbreak of COVID-19 epidemic in China, detection kits using isothermal amplification have been developed. A NAT kit for COVID-19 based on the isothermal amplification was approved on 22 February, featuring rapid detection, simple screening and intuitive visualization. Nevertheless, isothermal amplification may contribute to a false positive signal. In addition, experimental design of primers used in isothermal amplification is complicated, and long fragments cannot be

amplified, which significantly limit its application in detecting COVID-19.

Nucleic acid mass spectrometry

As a novel soft-ionization mass spectrometry, nucleic acid mass spectrometry integrates microfluidic chip and MALDI-TOF-MS (Matrix-Assisted Laser Desorption/Ionization time of flight Mass Spectrometry) to acquire maximal information and precise diagnosis. Featuring high sensitivity, high throughput sequencing and simple procedures, nucleic acid mass spectrometry is suitable for pathogens identification of respiratory infectious diseases [21]. Base-specific cleavage method combined MALDI-MS has been applied in genotype identification of hepatitis B virus. Ganova-Raeva et al. confirmed that the reliability and general efficiency of this novel method is prior to the traditional sequencing technology [39]. MALDI-MS is significantly superb in the large-scale analysis. Based on TOF-MS, NAT kits for common respiratory viruses (e.g. influenza A and B viruses) and COVID-19 have been developed with the detection limit of 100 copies/ml. However, nucleic acid mass spectrometry is difficult to be widely applied because of its high demands for microarrays, equipment, working condition and operational skills. Capillary electrophoresis allows simultaneous detection of several respiratory viruses. Nevertheless, it is rarely reported in the application of COVID-19 detection, such as the GenomeLab GeXp Genetic Analysis System [39].

Gene editing

CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9) system is a simple and effective gene editing technology. It has been extensively applied in gene editing, nucleic acid imaging, transcription regulation, gene detection, disease diagnosis and animal model establishment since its first emergence in 2012. In practice, CRISPR/Cas9 system has been rapidly developed for nucleic acid detection. Cas9 is an RNA-guided DNA endonuclease enzyme that is able to site-directly modify, edit genes and transcript products [40]. Leist et al. suggested that CRISPR-Cas9 system can be used for detecting MERS-CoV [41]. Nguyen et al. consistently demonstrated the application of CRISPR-Cas13d system in detecting ORF1ab and Spike in COVID-19 [42]. After recognition of COVID-19 by the guide RNAs targeting ORF1ab and Spike, Cas13 cleaves the target DNAs, followed by detection using LAMP or biosensors. Gootenberg et al. designed a detection platform termed SHERLOCK that combines isothermal pre-amplification with Cas13 to detect COVID-19, which is published on the website of McGovern Institute for Brain Research [43]. SHERLOCK highlights its potential as a multiplex, portable, rapid and quantitative detection of nucleic acids. Similar NAT kits have been developed by Chinese enterprises, but their applicability has not been verified in the clinic practice. In addition, the complicity in guide RNA design and multiuse primers may result in false negative signals owing to the low specificity and off-target effect.

Conclusions

Conventional methods, including conventional culture and serological test, are not suitable for detecting the newly emerged COVID-19. NAT, therefore, is firstly used for COVID-19 diagnosis. The summary of NATs was shown in **Table 1**. It is of great clinical significance in diagnosis, effective monitoring and prevention management of COVID-19. Nevertheless, clinical assessment of these NAT kits is lacked since the pathogenesis of COVID-19 remains largely unclear. Notably, standardization of laboratory test, including quality control and result interpretation, should be well concerned. Asymptomatic infection and false negative signals of COVID-19 should be well coped with, to ensure the effectiveness and accuracy of nucleic acid detection. So far, we can detect COVID-19 based on the genome sequences. More strides are required to clarify the origin, transmission routes and disease spectrum of COVID-19, aiming to develop effective therapeutic drugs and vaccines.

Acknowledgements

This work was supported in part by 333 high level talents training project of Jiangsu Province and Six talent peaks project in Jiangsu Province (WSN-121).

Disclosure of conflict of interest

None.

Table 1. A summary of NATs for 2019-nCoV

Method	Sensitivity	Specificity	Detection of throughput	Advantage	Disadvantages
RT-PCR	Low	High	Low	Both probes targeting ORF1ab and Nucleocapsid or Envelope of 2019-nCoV are detected. Positive signals of both ORF1ab and Nucleocapsid or Envelope can be confirmed as positive case of 2019-nCoV.	A negative signal cannot exclude the possibility of 2019-nCoV infection, and any reasons leading to false negative results should be considered.
dPCR	High	High	Low	Absolute quantification of nucleic acid amounts with high accuracy and reproducibility. Independences on threshold per cycle, internal controls and standard curves.	Limited use in a small number of high-level hospitals because of the strong dependence on the equipment and expensive cost.
mNGS	High	High	High	Sequences in other regions of 2019-nCoV are contained besides signals of ORF1ab and Nucleocapsid. False negative signals resulted from mutations outside the regions that RT-PCR detect are prevented. More sequence information is obtained to assemble genome sequences, trace evolution resource and predict virus transmission.	mNGS is not suitable in the large-scale and quick diagnosis of 2019-nCoV because of complicated procedures and long assay time.
Isothermal amplification	High	High	High	Rapid and accurate detection of RNAs in combination with CRISPR systems. Clinical application in detecting pathogens (e.g. ORF and Nucleocapsid proteins in MERS-CoV).	Limited use in detecting 2019-nCoV because of complicated design of primers and difficulties in long fragment amplification.
Nucleic acid mass spectrometry	High	High	High	Based on TOF-MS, NAT kits can be used for detecting common respiratory viruses (e.g. influenza A and B viruses) and 2019-nCoV with the detection limit of 100 copies/ml.	It is difficult to be widely applied because of its high demands for microarrays, equipment, working condition and personnel qualification.
Gene editing	High	High	Low	Multiplex, portable, rapid and quantitative detection of nucleic acids.	The complicity in guide RNA design and multiuse primers may result in false negative signals owing to the low specificity and off-target effect.

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