Review of Progress in New-Coronavirus Detection and Vaccine Research

Tongtong Liu*

Chengdu Jiaxiang Foreign Languages School, Chengdu, Sichuan Province, China

Abstract. Since December 2019, new coronaviruses have been spreading in the population with rapid transmission and high incidence, which are not easy to prevent. In this paper, we compiled and summarized the current status and characteristics of new-coronavirus detection and vaccine development by reviewing literature databases such as China Knowledge Network and PubMed. Through the principle of action analysis, we focused on comparing the pros and cons of various routes of new coronavirus detection, and the advantages and disadvantages of several different types of new coronavirus vaccines. Through the comparison, we found that 1. different detection routes target different populations, and the integrated application of multiple detection routes can lead to more accurate conclusions about virus detection. 2.several of the new crown vaccines on the market do not have the same mechanism of action, and you will need to choose according to your specific needs.3. in addition, the mRNA NCC vaccine is a great advancement of NCC vaccines in China. With the advantages of a development cycle and low side effects, the mRNA vaccine will become an important trend in future vaccine development.

Keywords: Novel coronavirus, Novel coronavirus pneumonia, Detection, Vaccine.

1. Introduction

In December 2019, a global outbreak of novel coronavirus pneumonia occurred. The number of infected patients has increased dramatically. Coronaviruses belong to the subfamily Coronaviridae and subfamily Orthocoronaviridae of the order Chaetomaviridae, and in 2011, the International Committee on Classification of Viruses classified coronaviruses into four genera: α, β, γ, and δ coronaviruses [1, 2]. The viruses of the genus Coronavirus are RNA viruses with capsids and linear single-stranded positive-stranded genomes, which are a large group of viruses widely found in nature. Coronaviruses infect only vertebrates. The genome is approximately 27-32 kbp in length, making it the largest genome of any RNA virus known. Coronaviruses that can infect humans include COVID-19, HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1, SARS-CoV, and MERS-CoV[3-5]. Human coronavirus infections are globally distributed, with almost 100% of children infected with HCoV-229E and NL63 in early childhood. The replication mechanism is unique, with a high frequency of homologous RNA recombination, resulting in the emergence of new mutant strains[6].

The RNA gene chain of the new coronavirus is a chain of RNA complexed in the N protein, consisting of 29,891 (about 30,000) nucleotides in tandem. Among them, nucleotide G and nucleotide C account for about 40%. The main function of the RNA gene chain is to preserve the genetic code of the new coronavirus for the production of the next generation of viral particles. The RNA gene chain contains the following five gene fragments: the S protein gene, which expresses the amino acid sequence of S protein, the E protein gene, which expresses the amino acid sequence of E protein, M protein gene, which expresses the amino acid sequence of M protein, and N protein gene, which expresses the amino acid sequence of N protein. Other genes for directing the replication process of the next generation of viral particles[7].

The S protein contains two conserved structural domains of interest, the spike receptor binding domain, and the coronavirus S2 glycoprotein. The main function of the S2 glycoprotein is to bind to the angiotensin-converting enzyme 2 (ACE2 enzyme) on the surface of human cells, allowing coronavirus particles to fuse to the cell interior, replicate, and produce more next-generation virus particles. After coronavirus enters the human body, it downregulates ACE2 levels, leading to a decrease in ACE2 levels in the lung, while ACE is not affected[8]. The imbalance between ACE2 and ACE in the lung and the increase in Ang II levels lead to excessive activation of AT1a receptors in the lung, resulting in increased capillary permeability in the lung, followed by pulmonary edema and induced dry cough, as well as increased inflammatory response and apoptosis, accelerating lung injury[9].

* Corresponding author: 2665494141@qq.com

Figure 1. Structural comparison between 2019-nCoV S and SARS-CoV S

In addition to their strong infectious and curative abilities, new coronaviruses are particularly susceptible to mutations because they are RNA viruses. nCoV genomes are abundant in patients, and the short replication cycle and high replication level of RNA viruses produce a large number of RNA virus genomes containing different mutations every day. All this contributes to the extremely high genetic diversity and variability of RNA viruses. Not to mention that environmental mutagenic factors (radiation, chemicals that modify bases, specific bases or base analogs, etc.), host RNA editing enzymes (adenosine deaminase, etc.), and other factors can further increase the mutation rate of RNA viruses. After mutation, viruses can enhance their infectivity, curative ability, and infectiousness by increasing the interactions between protein bases.

Because of the rapid mutation of viruses, their ability to spread quickly, and their infectiousness, it is imperative and trendy to find suitable and effective detection tools and develop more efficient vaccines. In this paper, we summarize the current types of new coronavirus detection methods and vaccines, comparing the differences between the various types in terms of principles, summarizing the advantages and disadvantages, and looking at various options with a developmental perspective to obtain better detection methods and more effective new coronavirus vaccines.

2. Current status of neo-coronavirus detection

Because of the insidious and widespread nature of neo-coronavirus transmission and the large number of people involved in being tested, it is important to be particularly flexible in choosing neo-coronavirus detection methods to achieve a combination of general screening and focused testing in the hope that the conclusion will be more rapid and accurate.

2.1 q-RT-PCR

The full name is real-time fluorescent reverse transcription polymerase chain reaction. It is a method that combines reverse transcription of RNA and polymerase chain amplification of cDNA, adds fluorescent groups to the PCR reaction system to monitor the whole PCR process in real time using fluorescent signal accumulation, and finally quantifies the unknown template by standard curve[10, 11].

The samples tested are pharyngeal swabs, nasal swabs, or alveolar lavage fluid, and the samples are first inactivated after they are taken. Inactivating the virus causes damage to the higher structure of the viral protein and the protein is no longer physiologically active, so it loses its ability to infect, cause disease and reproduce, but routine inactivation does not affect the primary structure of the viral protein, meaning that the sequence of the viral protein remains unchanged. Inactivation can be performed by physical means, such as heating, because most viruses are intolerant of high temperatures, such as the new coronavirus can be inactivated in 30 minutes at 56 degrees C. In addition, chemical methods, such as 75% alcohol, chlorinated disinfectants, or peroxyacetic acid, can be used to kill the virus[12]. In addition, UV irradiation and X-rays also have the effect of inactivating the virus. Cell lysis techniques are performed afterward. Cell lysis is a technique that uses external forces to disrupt the cell membrane and cell wall to release the cell contents, including the target product components. After lysis, RNA extraction and purification are performed. Extraction is generally performed by the Trizol method. This method maintains the integrity of the RNA during cell fragmentation and lysis. After extraction, the RNA can be separated from the protein and DNA, resulting in an RNA sample. The following reverse transcription process is used to synthesize complementary DNA using the RNA as a template, and the process commonly used for reverse transcription of laboratory templates is as follows[12].

1). Mix each component gently before use, then centrifuge at 2000rpm for the 20s.
2). Take a 0.2ml centrifuge tube that is sterilized and free of nucleases, and add 2~5μg RNA nμL in turn.
3). Hold at 65℃ for 5min, then ice bath for 5min.
4). Add the following components to the 0.2ml centrifuge tube in step 3.
RNase inhibitor (40u/μL) 0.5μL
10×M-MLVReactionBuffer2μL
DTT (200mM) 1μL
Reverse transcriptase (M-MLV) 1μL
5). after gentle mixing, then centrifuge at 2000rpm for 20s.
6). Hold at 37℃ for 1hr, then 70℃ for 15min.
7). The above products can be immediately carried out in the next PCR reaction or stored at -20℃.

The DNA template required for PCR was obtained by the above steps. After adding the PCR components according to the following system, the reaction is carried out according to the process of DNA amplification in vitro. DNA pre-denaturation-denaturation-annealing-extension.The general q-RT-PCR reaction detects ORF1a/b, E gene, and M gene in the neo coronavirus genome. Because the ORF1ab gene is more specific, while E and N genes are relatively conserved within the coronavirus itself[5, 13].

2.2 Neo-coronavirus antigen and antibody detection

It is a colloidal form of gold-labeled biological macromolecules, and in the PVC material test paper is fixed, leaving the sample addition hole, set up the detection line and quality control line, so that the sample in the sample addition hole plus, and then add a few drops of liquid medium, let the liquid flow to the other end of the test paper absorbent pad for flow, the test paper for chromatography, generally 10~15 minutes, and so on the test paper after the immune response occurs, you can see whether there is a red band appearing on the location of colloidal gold aggregation, visible to the naked eye, so that the test results will be known[14].

The principle of antigen and antibody testing is different, the antigen test detects the virus itself and is a direct test. The antigen test is for the antigen of the new coronavirus. The new coronavirus gene encodes multiple structural proteins, such as the N protein and S protein, which include multiple antigenic epitopes. Using the principle of specific binding of antigen to antibody, the presence of antigen can be detected by antibody, thus directly proving whether the sample contains the new coronavirus antigen[5].

The antibody test detects antibodies in the blood produced by the body after immunization, which can be subdivided into IgM antibody tests for pre-infection and lgG antibody tests for the later stages of treatment. When a new coronavirus first infects the body, it activates mainly B cells to produce specific antibodies against the new coronavirus. IgM is the earliest antibody that appears in the early and middle stages of the disease and is no longer detectable after a period of recovery from treatment. lgG is present from about a week after the onset of the disease and can be used not only to diagnose the disease but also to determine whether the infection has occurred in the past[15].

2.3 Genetic sequencing of neo coronavirus

At present, screening for neo-coronavirus is mainly performed by real-time fluorescence quantitative PCR, while the variety of mutations in neo-coronavirus variants-more than 7,000 species are found in India alone-cannot be detected by RT-PCR methods. To address this challenge, the detection of neo-coronavirus mutant strains by Sanger sequencing has become a breakthrough.

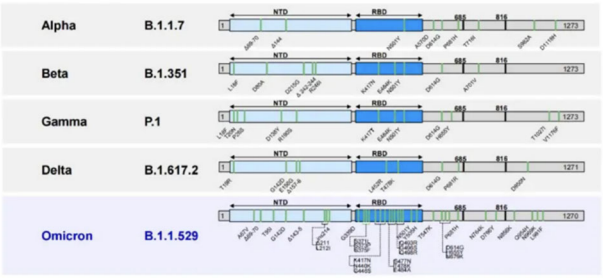

Figure 2. Mutations in the Spike gene at different positions in mutant strains

The mutation of neo-coronavirus is mainly the mutation of Spike protein (S protein), which is an important site for neo-coronavirus to bind to the receptor[9]. Figure 2 shows that the five major mutants Alpha, Beta, Gamma, Delta and Omicron that have emerged from the current evolution of the new crown strains are due to mutations at different positions in the gene encoding Spike, and where the mutations are located[7]. The basic principle of sanger sequencing is to use a DNA polymerase to extend the primers bound to the template of the sequence to be sequenced. The basic principle of sanger sequencing is to use a DNA polymerase to extend the primers bound to the template of the sequence to be sequenced until a strand termination nucleotide is incorporated. Each sequence assay consists of a set of four separate reactions, each containing all four deoxynucleotide triphosphates (dNTP), mixed with a limited amount of a different dideoxynucleotide triphosphate (ddNTP). However, the lack of the 3-OH group required for the extension of ddNTP causes the extended oligonucleotide to terminate selectively at G, A, T, or C. The termination point is determined by the corresponding double deoxy in the reaction. The relative concentration of each dNTPs and ddNTPs can be adjusted so that the reaction yields a set of chain termination products that are several hundred to several thousand bases long. They have a common starting point but terminate at different nucleotides and can be separated by high-resolution denaturing gel electrophoresis of fragments of different sizes, which can be detected by X-ray film radiolucent autoradiography or non-isotope labeling after gel processing. The experiment contains: the target fragment, four deoxyribonucleotides (dNTP), DNA polymerase, primers, and in addition, in each system, a deoxyribonucleotide (ddNTP) with 3'-OH removed, respectively.

In addition to this, whole genome sequencing is also a method to detect viruses. Whole genome sequencing is the genome sequencing of an individual species with an unknown genome sequence. However, it is generally more expensive and is usually used when identifying new strains[16].

3. Current status of research on new crown pneumonia vaccines

3.1 Adenovirus vector vaccine

Adenovirus vector vaccine is a vector vaccine that uses adenovirus as the active ingredient. The antigenic gene of the new coronavirus is also extracted and implanted into the body of the castrated adenovirus by genetic modification technology. The recombinant adenovirus is the core component of the vaccine. The genes expressed early in the adenovirus DNA replication cycle, called early genes, are mainly four, in order of expression: E1, E2, E3, and E4[5]. To prepare a self-inactivating recombinant adenovirus, the E1 gene is deleted, making the recombinant adenovirus a safe vehicle for gene transport. To increase packaging capacity, the E3 gene is also deleted at the same time, as the E3 gene product enhances the host's immune response and is not important

for virus production. The adenovirus genomic sequence with the missing E1 and E3 genes was cloned onto a plasmid to construct an adenoviral vector[17].
The adenovirus vector vaccine mimics the replication pattern of a normal virus when it invades the body, and protection can be achieved with as little as 1 dose. However, unlike normal viral infections where the virus itself replicates in large quantities, the vector vaccine only replicates a large amount of antigen, which stimulates a strong immune response[5]. The virus vector itself does not cause disease or replicate itself, so it is theoretically safe. And the same viral vector can be used to deliver different genetic material, which can greatly speed up the process of vaccine development. However, theoretically, the effectiveness of the vaccine may be weakened if the body has antibodies to the chosen viral vector itself. The technology is newer, and both the AstraZeneca vaccine and the Johnson & Johnson New Crown vaccine, which also use adenoviral vector technology, have recently been associated with rare but serious blood clots in a very small number of people after vaccination, and experts have not yet been able to identify the specific cause of the disease, but believe it may be related to the vaccine[18].

3.2 Inactivated and attenuated vaccines

Inactivated vaccines are cultures of bacteria, viruses, or rickettsiae that have been chemically or physically inactivated. It is made to completely lose the pathogenicity to the original target organ while preserving the corresponding antigen's immunogenicity. This is a traditional vaccine production method that has been proven over a long period. It is theoretically safe because the germs used in the vaccine are inactivated (killed) and no longer cause disease. However, because the germs used are inactive and less immunogenic, inactivated vaccines usually require the addition of an adjuvant (adjuvant) to help stimulate the body's immune system response, and two to three doses are required to achieve protection[18].
Live attenuated vaccines are a class of vaccines in which the structure of the A subunit (virulent subunit) is changed and toxicity is reduced after formaldehyde treatment of the pathogen, but the activity of the B subunit (conjugate subunit) remains unchanged, and antigenicity is maintained. Inoculation into the body does not cause the development of disease, but the pathogen can trigger the body's immune response and stimulate the body to produce specific memory B cells and memory T cells. The effect is to obtain long-term or lifelong protection. Live microorganisms are used, which can act in the body for a long time and induce a strong immune response. Since live microorganisms have the property of repopulating, it is theoretically possible to achieve a satisfactory immune effect with only one vaccination[19]. Moreover, it may cause horizontal transmission, expand the immunization effect and enhance the herd immunity barrier. No adjuvant is needed for production, and the production process generally does not require concentration and purification and is inexpensive. However, live attenuated vaccines generally retain residual virulence, which may induce serious diseases in some individuals (such as immunodeficient individuals)[20].

3.3 mRNA vaccine

mRNA vaccines produce mRNA fragments of specific antigenic proteins and are more direct than viral vector vaccines in that they are delivered directly into cells without a viral vector, but rather by encapsulating mRNA in lipid nanoparticles, which then direct the cells to produce antigenic proteins and stimulate an immune response. mRNA vaccines were developed based on the mechanism by which mRNA works. As an in vitro transcribed nucleic acid vaccine, small harmless fragments of the virus are presented to immune cells to "learn" and "rehearse", teaching them how to recognize and attack the virus, so that the next time a new coronavirus comes, a specific immune response can be rapidly generated, thus preventing the virus from replicating and spreading. This prevents the virus from replicating and spreading in the body[4].
The mRNA vaccine has a short development cycle, which can shorten the overall process to 0.8-1.5 years with the support of an epidemic, and there is no risk of infection. mRNA vaccine does not contain intact virus compared to traditional inactivated and live attenuated vaccines, so there is no risk of virus infection in human organisms. mRNA vaccine can induce both humoral immunity and cellular immunity in the human body, which has a dual immune effect. has a dual immune effect, and the immunity is long-lasting. However, it is very difficult to find a sequence that is most suitable for drug generation. Sequence structure determines the quality of the vaccine, including antigenic protein structure, immunogenicity, and stability.

4. Conclusion

For the detection of neo-coronaviruses, RT-PCR is a high-throughput and low-cost method for large-scale pre-screening, which can detect the entire infection stage, but the sample processing time is long, and the pre-processing of samples requires a high-level of laboratory safety and is relatively complicated. Antigen detection methods can also be developed into high-throughput methods, fast and accurate. However, the cost of the experiment is relatively high, and some antibodies will be used. Therefore, antigen testing is more suitable for small-scale screening and diagnosis. Antibody testing is an indirect test, with a high probability of false positives and different types of antibodies, and is more suitable for individual testing[19]. Sanger sequencing and whole-genome sequencing of viruses are suitable for the identification of viruses and viral variants, and are more accurate because they measure the composition of each base[10, 21].
In addition, we have the CRISPR assay, which is the newest technology in biology and medicine this year, with much of the research and development in the early stages. However, this technology is only involved in laboratory research and has not yet been promoted for large-scale use, but CRISPR technology is also a key direction for future development[22].

For the new crown vaccine research and development to the book, several vaccines currently exist, although they have been monitored for safety, vaccine efficacy, and adverse effects are still not fully resolved. In comparison, mRNA vaccines with fewer side effects may be the future direction of development, but there are still many technical barriers in China in this regard, and we hope to make a breakthrough in the coming time.

References

1. Sampath, V., et al., Vaccines and allergic reactions: The past, the current COVID-19 pandemic, and future perspectives. Allergy, 2021. 76(6): p. 1640-1660.
2. Rai, P., et al., Detection technologies and recent developments in the diagnosis of COVID-19 infection. Appl Microbiol Biotechnol, 2021. 105(2): p. 441-455.
3. Guarner, J., Three Emerging Coronaviruses in Two Decades. Am J Clin Pathol, 2020. 153(4): p. 420-421.
4. Abdelrahman, Z., M. Li, and X. Wang, Comparative Review of SARS-CoV-2, SARS-CoV, MERS-CoV, and Influenza A Respiratory Viruses. Front Immunol, 2020. 11: p. 552909.
5. Chen, Y., Q. Liu, and D. Guo, Emerging coronaviruses: Genome structure, replication, and pathogenesis. J Med Virol, 2020. 92(4): p. 418-423.
6. Alanagreh, L., F. Alzoughool, and M. Atoum, The Human Coronavirus Disease COVID-19: Its Origin, Characteristics, and Insights into Potential Drugs and Its Mechanisms. Pathogens, 2020. 9(5).
7. Chaudhary, J.K., et al., Insights into COVID-19 Vaccine Development Based on Immunogenic Structural Proteins of SARS-CoV-2, Host Immune Responses, and Herd Immunity. Cells, 2021. 10(11).
8. Lu, R., et al., Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet, 2020. 395(10224): p. 565-574.
9. Yang, M., et al., Structural Basis of a Human Neutralizing Antibody Specific to the SARS-CoV-2 Spike Protein Receptor-Binding Domain. Microbiol Spectr, 2021. 9(2): p. e0135221.
10. Alsharif, W. and A. Qurashi, Effectiveness of COVID-19 diagnosis and management tools: A review. Radiography (Lond), 2021. 27(2): p. 682-687.
11. Ai, T., et al., Correlation of Chest CT and RT-PCR Testing for Coronavirus Disease 2019 (COVID-19) in China: A Report of 1014 Cases. Radiology, 2020. 296(2): p. E32-e40.
12. Gaunt, E.R., et al., Epidemiology and clinical presentations of the four human coronaviruses 229E, HKU1, NL63, and OC43 detected over 3 years using a novel multiplex real-time PCR method. J Clin Microbiol, 2010. 48(8): p. 2940-7.
13. Sidiq, Z., et al., Benefits and limitations of serological assays in COVID-19 infection. Indian J Tuberc, 2020. 67(4s): p. S163-s166.
14. Faiq, M.A., B-cell engineering: A promising approach towards vaccine development for COVID-19. Med Hypotheses, 2020. 144: p. 109948.
15. Mistry, P., et al., SARS-CoV-2 Variants, Vaccines, and Host Immunity. Front Immunol, 2021. 12: p. 809244.
16. Su, S., et al., Epidemiology, Genetic Recombination, and Pathogenesis of Coronaviruses. Trends Microbiol, 2016. 24(6): p. 490-502.
17. Anand, U., et al., Potential Therapeutic Targets and Vaccine Development for SARS-CoV-2/COVID-19 Pandemic Management: A Review on the Recent Update. Front Immunol, 2021. 12: p. 658519.
18. Sharma, O., et al., A Review of the Progress and Challenges of Developing a Vaccine for COVID-19. Front Immunol, 2020. 11: p. 585354.
19. Melo-González, F., et al., Recognition of Variants of Concern by Antibodies and T Cells Induced by a SARS-CoV-2 Inactivated Vaccine. Front Immunol, 2021. 12: p. 747830.
20. Zhang, Y., et al., Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18-59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. Lancet Infect Dis, 2021. 21(2): p. 181-192.
21. Ochani, R., et al., COVID-19 pandemic: from origins to outcomes. A comprehensive review of viral pathogenesis, clinical manifestations, diagnostic evaluation, and management. Infez Med, 2021. 29(1): p. 20-36.
22. Sreepadmanabh, M., A.K. Sahu, and A. Chande, COVID-19: Advances in diagnostic tools, treatment strategies, and vaccine development. J Biosci, 2020. 45(1).