ARTICLE @ VIROLOGY

Research Progress on Residual Exogenous DNA in mRNA Vaccines

*Danhua Zhao, Yanqiu Huang, and Shouchun Cao**

National Institutes for Food and Drug Control, Division of Arboviral Vaccines, Beijing 102629, P.R.China

ABSTRACT

mRNA vaccines exhibit several advantages, including a short development cycle, strong immunogenicity, and precise targeting. They hold significant potential for preventing infectious diseases and treating tumors. However, residual exogenous DNA from the production process is a critical quality control indicator affecting product safety. This article reviews recent advances in the field, focusing on the sources, risk assessment, and control methods of such residues. Studies have shown that these residues mainly originate from plasmid DNA and various production steps. The level of risk is closely associated with the amount of residual DNA and the length of the fragments. Long DNA fragments (>200 bp) carry a potential risk of causing insertional mutations and therefore represent a key focus in quality control, which requires particular attention. At present, a risk-based framework has been established within quality control systems. For infectious disease vaccines, China has set a stricter limit of ≤5 ng per dose compared with international standards. This standard aligns more closely with the public health protection needs in China and is considered essential. Detection technologies are primarily centered on digital droplet PCR, complemented by supplementary methods. This combined approach helps reduce errors inherent in any single method and improves the reliability of results. The optimization of template design, improvement of purification efficiency, and full-process quality control covering "raw materials – production process – final products" have provided technical support for residual control. Currently, there are still some problems in this field, including the absence of uniform detection standards, incompatibility between novel processes and quality control, and inconsistent standards among different countries. In the future, it will be necessary to drive innovation in detection technologies, achieve intelligent integration of processes and quality control, and refine relevant standards to establish a comprehensive management and control system. Only in this way can we achieve precise full-chain control and ensure the clinical application safety of mRNA vaccines as well as the sustainable development of the industry.

Article History: Submitted: 11/07/2025; Revised: 28/12/2025; Accepted: 25/02/2026

DOI:10.21092/jav.v15i1.123

Key Words: mRNA Vaccines; Residual Exogenous DNA; SARS-CoV-2; Detection Technology; Production Process; Quality Control; Standards.

Funding: The work is supported by the Youth Development Research Foundation of NIFDC (Grant No. *2024B1*).

* Corresponding author, Major in Immunology, E-mail: caosc@nifdc.org.cn

Copyright©2012-2026 Published by Hongkong Institute of Biologicals Standardization Limited.
All rights reserved.

Introduction

Following the outbreak of the COVID-19 pandemic in 2019, mRNA vaccines became a major focus in global vaccine research and development due to their distinct molecular mechanism of action[18,22]. After being synthesized in vitro, mRNA is delivered into the human body via a delivery system. Once inside the human body, the mRNA directly directs the production of the antigen and elicits a specific immune response. This approach significantly accelerated vaccine development and production timelines as it does not rely on traditional virus cultivation or genomic integration[2,6]. Given its flexibility and high efficiency, mRNA technology not only holds broad potential for preventing infectious diseases but also offers a novel approach for cancer therapy[6,25].

However, the production of mRNA vaccines requires the use of plasmid DNA as a template. Even after purification, small amounts of exogenous DNA fragments may still remain in the final product[1,11]. Such residual fragments may not only cause abnormal immune responses but also integrate into the human genome and induce gene mutations[23,27]. Therefore, controlling the quantity and fragment length of these residues has become a key indicator for evaluating vaccine quality[29].

In recent years, researchers at home and abroad have carried out extensive studies focusing on the detection technologies, control strategies, and quality standards for residual exogenous DNA[3,19,30]. The World Health Organization (WHO) has issued guidelines relevant to mRNA vaccines, providing a reference basis for global vaccine quality control[30]. Researchers in China have identified key quality control points tailored to the specific characteristics of local vaccine production.

Furthermore, they have also established a quality control concept in which processes, detection methods, and standards coordinate with each other, a concept that better aligns with the actual situation of vaccine manufacturing in China[29].

Sources and Potential Risks

1. Sources of Residues

Exogenous DNA residue is a critical quality control point in the manufacturing process of mRNA vaccines, originating primarily from two stages. The first stage is residual DNA related to the plasmid DNA template, including incompletely digested circular or linearized plasmid fragments. Studies have confirmed that the degree of plasmid linearization directly influences the amount of residue. Optimizing linearization reaction conditions—such as adjusting enzyme activity, reaction temperature, and reaction time—can increase linearization efficiency to over 95%, thereby significantly reducing residual levels[1,15,24]. Additionally, optimizing plasmid vector sequences and removing redundant DNA fragments can further reduce the risk of template residues[4].

The second stage involves residual control during the purification steps of the manufacturing process. The digestion efficiency of DNase is critical; if efficiency falls below 99%, the residual amount increases significantly. Selecting highly specific DNase and optimizing the reaction system can achieve more thorough DNA degradation[3].Adopting the three-step purification method of "chromatography-ultrafiltration-chromatography" can stably control the residue level below 10 pg per dose, which meets international standards[24]. In addition, the production

environment and equipment may introduce trace contamination, but the proportion of such contamination is usually extremely low(<0.1%). Such contamination can be avoided by enhancing environmental monitoring and material control[24]. The emerging cell-free production systems reduce the required amount of plasmid template and, when combined with novel purification technologies, can control residuals below 5 pg per dose. This establishes a solid process foundation for setting more stringent quality standards [25].

2. Potential Risks

The safety risks of exogenous DNA residues are closely associated with the residual amount, fragment length, and sequence characteristics. These risks are mainly reflected in three aspects. First, intact plasmid DNA may carry functional elements such as antibiotic resistance genes and promoters. If these elements enter human cells, they may lead to aberrant gene expression and increase the risk of spreading antibiotic resistance. Special attention should be paid to this risk in immunocompromised individuals [16,20].

Second, unmethylated CpG sequences within residual DNA fragments can activate the innate immune response in humans via the TLR9 pathway, leading to inflammatory reactions. The short-term adverse reactions of some vaccines are associated with this inflammatory response: the higher the residue level, the stronger the inflammatory response[23,26]. Third, longer DNA fragments (>200 bp) carry a potential risk of random integration into the human genome. It has a much higher probability of inducing insertional mutagenesis than short-fragment DNA, making it a core focus of quality control[13,27].

Analysis of existing COVID-19 mRNA vaccines indicates that their levels of residual DNA comply with international standards, and no severe adverse events have been reported following short-term use[14]. However, their long-term potential risks still require validation through large-scale, long-term follow-up studies. Therefore, in quality control, it is essential to continuously and strictly monitor both the total residual DNA and the fragment length distribution, with particular attention to the risk associated with long DNA fragments (>200 bp) [20,23,29].

Quality Control Standards

The quality control standards for residual exogenous DNA constitute a systematic framework that integrates risk assessment, manufacturing processes, and detection technologies, serving as the central basis for ensuring vaccine product safety[19,30].

1. Core Components of Quality Standards

The core components of quality standards include residual DNA limits, fragment length control, and detection method requirements. Based on risk assessment, nations have established quality control frameworks that are consistent in principle but vary in specific limits [27,30].

In terms of residual DNA limits, a global risk-based tiered control system has been established. In line with the needs of infectious disease prevention and leveraging its own technological strengths, China has proposed a more stringent standard of ≤5 ng per dose for mRNA vaccines against infectious diseases. This standard has been incorporated into the relevant draft guidance, reflecting the principle of "prioritizing risk control"[29,30]. In contrast, both China's general standards and other major international guidelines, such as those from the WHO, set the limit at ≤10 ng per dose [27,30]. Furthermore, European and American standards additionally stipulate that DNA residues must not exceed 1 ng per μg of mRNA, and require the provision of data with a

Copyright©2012-2026 Published by Hongkong Institute of Biologicals Standardization Limited.
All rights reserved.

95% confidence interval to enhance the reliability of statistical results [16,23].

In terms of fragment length control, it is widely recognized within the industry that longer DNA fragments (>200 bp) pose a higher potential risk of insertional mutagenesis. Therefore, WHO as well as European and American standards require that over 90% of residual DNA fragments be shorter than 200 bp[16]. For vaccines targeting infectious diseases, China has established more detailed guidelines, recommending that longer fragments (>200 bp) should not exceed 1 ng per dose and should constitute no more than 20% of the total residual DNA[29]. Research data indicate that DNA fragments longer than 500 bp carry an insertional risk more than 10 times greater than those below 200 bp, providing a key scientific basis for fragment length control[16].

In terms of detection method requirements, standards specify clear performance criteria for detection methods, typically including a limit of detection (LOD) ≤0.01 ng/μL, a recovery rate between 90% and 110%, and validation of specificity to exclude matrix interference[1,28]. To enhance the reliability of detection results, the use of orthogonal detection methods is recommended. This approach combines techniques based on different principles-such as digital droplet PCR (ddPCR) and high-performance liquid chromatography (HPLC)-for cross-verification[14,30]. This combined methods effectively reduces errors associated with any single method, thereby providing more robust and convincing data.

2. The Role of Process Optimization in Standard Implementation

Continuous optimization of production processes serves as the technical foundation for implementing stringent quality control standards, with such optimization being applied throughout upstream and downstream manufacturing steps[3,24].

2.1 Upstream Preparation Stage

Optimization of plasmid backbone design, such as removing non-essential sequences while improving linearization efficiency, can reduce the residual levels of intact plasmids or large DNA fragments at the source, thereby laying the foundation for subsequent compliance with standards[4].

2.2 In Vitro Transcription Stage

The use of high-purity linearized templates-with an A_{260}/A_{280} ratio between 1.8 and 2.0—and optimization of reaction conditions are critical. Studies indicate that for every 10% increase in transcription efficiency, residual DNA in the final product can be reduced by 15–20% [15].

2.3 Purification Stage

The purification process is central to the deep removal of residual DNA. Combining multiple purification techniques-such as ion-exchange chromatography, gel filtration, and ultrafiltration-enables the efficient separation of DNA fragments. Scale-up production data confirm that this integrated approach can reduce DNA residues from an initial level of 100 ng per dose to below 10 pg per dose, thereby meeting the most stringent standards[24,25]. For mRNA vaccines delivered via lipid nanoparticles (LNPs), process optimization focuses on adjusting lipid formulations and controlling preparation parameters to minimize DNA encapsulation, thereby enhancing the efficiency of downstream purification[27].

Full-Process Implementation of the Quality Control System

The effective implementation of quality control standards relies on a closed-loop management system that covers the entire workflow, from raw materials and production processes through to the final product[3,19].

Quality control of raw materials serves as the first line of defense, focusing on ensuring the high purity (A_{260}/A_{280} ratio of 1.8–2.0) and low host cell DNA (HCD) residuals (<10 ng/μg) of the plasmid DNA template, thereby establishing favorable conditions for downstream manufacturing processes[24].

In-process quality control provides real-time assurance. The application of process analytical technology enables real-time monitoring of removal efficiency (e.g., requiring digestion efficiency ≥99.9%) or residual levels at critical steps such as DNase digestion and chromatographic purification, while allowing dynamic adjustment of process parameters[15,19,28]. Establishing a "process-quality" correlation model allows precise control of critical process parameters. Cases have demonstrated that such a model can control inter-batch variability of residues within 5%, highlighting its value for broader adoption[3].

Final product quality control serves as the basis for vaccine release, requiring the use of validated orthogonal methods to strictly quantify both the total DNA residue and fragment length distribution. For example, mRNA vaccines against infectious diseases must simultaneously meet the dual criteria of total residue ≤5 ng/dose and long-fragment DNA ≤1 ng/dose[29]. Currently, quality control systems are evolving toward intelligent development, integrating real-time monitoring data with machine learning algorithms to predict process impacts, enable proactive optimization, and dynamically elevate the overall quality control standard.

Detection Technologies

The detection of exogenous DNA residuals is a core technology for the implementation of quality control standards. Currently, the industry has established a detection system centered on PCR technology and complemented by multiple methodologies. The sensitivity, specificity, and applicable scenarios of each technique are well-aligned with quality control requirements [11,28].

1. PCR-Based Detection Technologies

PCR technology, characterized by high sensitivity and strong specificity, serves as the primary method for detecting DNA residuals. It mainly includes three types[9-11]. Two-step quantitative RT-PCR can indirectly assess DNA residuals, with a limit of detection around 0.05 ng/μL, making it suitable for preliminary screening and batch testing during production[9]. However, it is susceptible to interference from RNA impurities and inhibitors, and lacks sufficient specificity for detecting short DNA fragments, which prevents it from meeting the stringent quality control requirements for finished infectious-disease vaccines. Therefore, it is primarily employed as a supplementary tool for in-process quality control[1,10].

Digital droplet PCR partitions the reaction mixture into tens of thousands of individual droplets, enabling single-molecule amplification. This significantly improves detection sensitivity and resistance to interference, establishing it as a core technology for finished vaccine product quality control[1,11]. Studies have shown that its limit of detection can reach 0.01 ng/μL, and it can effectively distinguish between linearized and circular plasmids. Its precision and accuracy meet stringent quality control requirements, with quantitative reliability notably superior to that of

Copyright©2012-2026 Published by Hongkong Institute of Biologicals Standardization Limited.
All rights reserved.

conventional quantitative PCR[1,11,16]. This technique is unaffected by matrix interference from mRNA, host cell proteins, and other impurities, and with specific primers and probes, it can achieve specific detection of long-fragment DNA[11,16].

Chip-based digital PCR builds upon droplet digital PCR by employing microfluidic chips to achieve precise partitioning and parallel detection of the reaction system, further enhancing detection efficiency and throughput[10,14]. It enables the division of the reaction mixture into over 100,000 nanoliter-sized partitions, reduces detection time by approximately 30%, and requires no complex sample pre-processing, making it suitable for real-time quality control during the manufacturing process[10]. With a limit of detection reaching 0.005 ng/μL, this method also supports multiplex detection, allowing simultaneous quantification of total residual DNA and long- fragment DNA in a single reaction[14].

2. Chromatographic Detection Techniques

High-performance liquid chromatography (HPLC) is an amplification-independent technique that quantifies nucleic acids by separating fragments based on molecular weight, providing distinct advantages for in-process quality control[15,28]. This method can directly measure residual linearized plasmid DNA with a limit of detection of approximately 0.1 ng/μL. It requires no nucleic acid extraction or amplification, thereby avoiding amplification bias, and is well-suited for monitoring linearization efficiency and preliminary purification effect[15]. However, it exhibits lower sensitivity than PCR-based methods and is therefore usually used in combination with PCR to establish a dual-verification system[28]. With further optimization, ultra-high-performance liquid chromatography (UHPLC) achieves a lower detection limit of approximately 0.05 ng/μL and reduces the analysis time to within 30 minutes, enhancing its utility in process monitoring[28].

3. Orthogonal Detection Methods

To improve the reliability of detection results and avoid the limitations of any single method, orthogonal detection has become an essential requirement in quality control systems, particularly for quality validation of vaccines prior to marketing[14,28]. For COVID-19 mRNA vaccines, for example, researchers employed four methods—digital droplet PCR, quantitative PCR, HPLC, and sequencing—which showed good concordance (relative deviation ≤8%), confirming that residual DNA levels met specifications[14]. This approach leverages the complementary strengths of different techniques: PCR excels at trace-level quantification, chromatography avoids amplification bias, and sequencing provides sequence and fragment length information. Together, they effectively reduce systematic error inherent to individual methods, providing a more comprehensive scientific basis for risk assessment[28,30]. For high-risk vaccines, orthogonal detection has become mandatory requirement, with common combinations including "ddPCR + HPLC" or "qPCR + NGS" .

4. Technical Development Trends

Detection technologies are expected to develop in three major directions in the future. First, by combining high sensitivity with high throughput, methods with detection limits reaching the picogram level and assay times shorter than one hour will be developed to meet the demands of rapid, large- scale quality control[28]. Second, sample preparation will be simplified through the development of direct detection methods without nucleic acid extraction, making them compatible with complex samples such as LNP-based delivery systems[10]. Third, multi-index combined detection will be realized, allowing simultaneous assessment of multiple critical quality

attributes-including residual DNA, mRNA integrity, host cell proteins, and LNP particle size-thereby improving overall quality control efficiency [28,29].

Table 1. Comparison of Detection Techniques for Residual Exogenous DNA

Detection Technique	Sensitivity	Specificity	Assay Time	Application
Real-Time quantitative PCR(qPCR)	Moderate (0.05 ng/μL)	Moderate	2-3 h	Preliminary screening and batch testing in production
Digital droplet PCR (ddPCR)	High (0.01 ng/μL)	High	3-4 h	Final product quantification; validation of new production processes
Chip-based digital PCR (cdPCR)	Very High (0.005 ng/μL)	High	1.5-2 h	Real-time in-process quality control; critical node monitoring
HPLC	Low (0.1 ng/μL)	Moderate	1 h	In-process quality control of linearized plasmid DNA; intermediate monitoring
UPLC	Moderate (0.05 ng/μL)	Moderate	0.5 h	Rapid in-process screening
Orthogonal detection (ddPCR + HPLC)	High (0.01 ng/μL)	Very High	4-5 h	Pre-market quality validation; quality control of high-risk vaccines

5. Summary and Outlook

5.1 Summary of Research Findings

Residual exogenous DNA is a critical quality attribute in the control of mRNA vaccines, with its origin being closely related to the production process. It may trigger risks including aberrant gene expression, immune interference and insertional mutagenesis, which necessitate a stringent quality control system[16,23,29]. From 2020 to 2025, significant progress has been made in this field, with key advancements centered on three areas. First, with respect to the sources and risk understanding of residual DNA, studies have clarified that residual plasmid template and inadequate process optimization are the main sources, and have validated the quantitative relationship among residual quantity, fragment length and associated risks. Long DNA fragments (>200 bp) pose a significantly higher risk than short ones, which provides a basis for tiered quality control standards[16,29]. Orthogonal testing has verified that residual DNA levels in existing marketed vaccines meet the quality control requirements, ensuring their safety and effectiveness in short-term clinical application[14]. Second, in terms of detection technology, the field has established a system centered on digital droplet PCR and complemented by multiple analytical methods. Chip-based digital PCR improves the efficiency of real-time in-process quality control during production, HPLC and UPLC are suitable for in-process screening, and orthogonal testing improves the reliability of results. Each technology has clearly defined applications, providing a solid foundation for implementing quality control standards[1,10,28]. Third, with regard to the compatibility between quality control standards and manufacturing processes, a global risk-based tiered framework has been established. China has introduced more stringent control limits for vaccines against infectious diseases than those in international guidelines, with dual control requirements[29,30]. Process optimization is deeply integrated with quality control; technologies

Copyright©2012-2026 Published by Hongkong Institute of Biologicals Standardization Limited.
All rights reserved.

such as three-step purification and cell-free production have achieved precise control of residuals, and a full-process quality system ensures effective standard implementation[3,24,25].

5.2 Research Gaps

Despite notable research progress, the field still faces three major challenges. First, there remains a lack of standardization in detection methods, as discrepancies in reagents, instruments, and protocols across laboratories and manufacturers lead to poor comparability of results. There is also no unified specification for fragment-length analysis[14,28]. Second, integration between manufacturing processes and quality control is insufficient. New technologies such as LNP delivery and continuous production require further optimization, and existing "process-quality" correlation models lack broad applicability[19,27]. Third, the harmonization of standards is lacking. Differences in residual DNA limits and method validation requirements across regions impede the global circulation of vaccines. In addition, the dynamic update mechanism of standards is imperfect, making it difficult to rapidly adapt to novel technological routes[30].

5.3 Future Perspectives

Building on existing research progress and technological trends, future efforts should focus on achieving breakthroughs in three key areas. In terms of detection technologies, the development of CRISPR-based nucleic acid assays and novel biosensors is needed to push the detection limit below 1 pg/μL[28]. Meanwhile, unified detection specifications and reference systems should be established, and integrated equipment developed to shorten the assay time to within 30 minutes[10]. In terms of manufacturing processes, cell-free production and purification technologies should be further optimized to consistently control residual DNA below 1 ng per dose[24,25]. Predictive models should be constructed based on process analytical technology (PAT), and synthetic biology techniques employed to reduce the difficulty of residual DNA control[3,19,25]. Regarding quality standards, residual DNA limits and fragment length specifications should be optimized based on long-term clinical data, establishing a classified and tiered quality control system tailored to different vaccine types and vaccinated populations[23,29]. International collaboration should be strengthened to promote the mutual recognition of globally unified detection methods and limit standards, thereby reducing trade barriers[30]. A dynamic updating mechanism for standards should be established to promptly incorporate requirements from novel detection technologies and production processes, ensuring the scientific validity and timeliness of standards[19].

Looking ahead, multidisciplinary collaboration is needed to refine the closed-loop system of “risk assessment-standard setting-process optimization-detection verification.” This will enable precise control of residual exogenous DNA throughout the entire chain, ensure the safety and efficacy of mRNA vaccines, and promote their sound development in infectious disease prevention and tumor therapy [6,25].

Funding

The work is supported by the Youth Development Research Foundation of NIFDC (Grant No. 2024B1). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests

The authors declare all financial and non-financial competing interests.

References

[1] Zhao D H, Liu X Y, Huang Y Q, et al. Establishment and validation of digital droplet PCR method for detecting residual template DNA in SARS-CoV-2 mRNA vaccine[J]. Chinese Journal of Biologicals, 2025, 38(4): 442-449. DOI:10.13200/j.cnki.cjb.004461.

[2] Ding M M, Han J X, Shen S X, et al. Research and application progress of mRNA vaccines and their technologies[J]. Biology Bulletin, 2025, 60(2): 1-5.

[3] Tang Q, Shi J R, Xing P D, et al. Research progress on production technology and quality control of mRNA vaccines[J]. Chinese Journal of New Drugs, 2024, 33(11): 1109-1114.

[4] Huang Y, Song Y. Analysis on key points of engineering design of mRNA vaccines[J]. Chemical and Pharmaceutical Engineering, 2023, 44(6): 32-35.

[5] Qin F M, Ren N, Cheng W Y, et al. mRNA vaccines for infectious diseases: principles and applications[J/OL]. Chinese Journal of Biotechnology, 2023 [2026-01-20]. https://link.cnki.net/urlid/11.1998.Q.20230724.1724.002.

[6] Wei D, Ma Y L, Wan F, et al. Research and application progress of mRNA vaccines [J]. Current Biotechnology, 2023, 13 (4): 492-498. DOI:10.19586/j.2095-2341.2023.0015.

[7] Li Y Z, Zhu J Y, Chen J J, et al. Modification strategies and their applications of mRNA vaccines[J]. Heilongjiang Animal Science and Veterinary Medicine, 2023(13): 36-43. DOI:10.13881/j.cnki.hljxmsy.2022.08.0185.

[8] Wang Y Y. Study on mRNA delivery vectors based on VLPs[D]. Shanghai: Shanghai University, 2023. DOI:10.27300/d.cnki.gshau.2023.000813.

[9] Ahlemeyer B, Colasante C, Baumgart-Vogt E. Analysis of the Level of Plasmid-Derived mRNA in the Presence of Residual Plasmid DNA by Two-Step Quantitative RT-PCR [J]. Methods and Protocols，2020，3 (2)：40. DOI:10.3390/mps3020040.

[10] Fan W, Zhao L, Yu L, et al. Chip-based digital PCR as a direct quantification method for residual DNA in mRNA drugs [J]. Journal of Pharmaceutical and Biomedical Analysis，2024，238：115837. DOI:10.1016/j.jpba.2023.115837.

[11] Kaiser S, Kaiser S, Reis J, et al. Quantification of objective concentrations of DNA impurities in mRNA vaccines [J]. Vaccine，2025，55：127022. DOI:10.1016/j.vaccine.2025.127022.

[12] Vieths S, Waibler Z, Schumann GG. Opting for the appropriate methodology for quantification of residual DNA impurities in mRNA vaccines [J]. Vaccine，2025，56：127186. DOI:10.1016/j.vaccine.2025.127186.

[13] Mead MN, Seneff S, Wolfinger R, et al. COVID-19 mRNA Vaccines: Lessons Learned from the Registrational Trials and Global Vaccination Campaign [J]. Cureus，2024，16 (1)：e52876. DOI:10.7759/cureus.52876.

[14] Achs A, Sedlackova T, Predajna L, et al. Systematic analysis of COVID-19 mRNA vaccines using four orthogonal approaches demonstrates no excessive DNA impurities [J]. NPJ Vaccines，2025，10 (1)：259. DOI:10.1038/s41541-025-01304-9.

[15] Leban M, Vodopivec Seravalli T, Hauer M, et al. Determination of linearized pDNA template in mRNA production process using HPLC [J]. Analytical and Bioanalytical Chemistry，2024，416 (10)：2389-2398. DOI:10.1007/s00216-024-05204-0.

[16] Speicher DJ, Rose J, McKernan K. Quantification of residual plasmid DNA and SV40 promoter-enhancer sequences in Pfizer/BioNTech and Moderna modRNA COVID-19 vaccines [J]. Autoimmunity，2025，58 (1)：2551517. DOI:10.1080/08916934.2025.2551517.

[17] Huang D, Li N, Dong X. Advances in mRNA vaccine research in the field of quality control [J]. Biologicals，2024，88：101799. DOI:10.1016/j.biologicals.2024.101799.

[18] Fang E，Liu X，Li M，et al. Advances in COVID-19 mRNA vaccine development [J]. Signal Transduction and Targeted Therapy，2022，7 (1)：94. DOI:10.1038/s41392-022-00950-y.

[19] Hu C, Bai Y, Liu J, et al. Research progress on the quality control of mRNA vaccines [J]. Expert Review of

Copyright©2012-2026 Published by Hongkong Institute of Biologicals Standardization Limited.
All rights reserved.

Vaccines，2024，23 (1)：570-583. DOI:10.1080/14760584.2024.2354251.
[20] Quan Y, Yang H, Li W, et al. mRNA vaccines: immunogenicity and quality characteristics [J]. Journal of Nanobiotechnology，2025，24 (1)：6. DOI:10.1186/s12951-025-03800-5.
[21] Graña C，Ghosn L，Evrenoglou T，et al. Efficacy and safety of COVID-19 vaccines [J]. Cochrane Database of Systematic Reviews，2022，12 (12)：CD015477. DOI:10.1002/14651858.CD015477.
[22] Baden LR, El Sahly HM, Essink B, et al. Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine [J]. New England Journal of Medicine，2021，384 (5)：403-416. DOI:10.1056/NEJMoa2035389.
[23] Banoun H. mRNA: Vaccine or Gene Therapy? The Safety Regulatory Issues [J]. International Journal of Molecular Sciences，2023，24 (13)：10514. DOI:10.3390/ijms241310514.
[24] Whitley J, Zwolinski C, Denis C, et al. Development of mRNA manufacturing for vaccines and therapeutics [J]. Translational Research，2022，242：38-55. DOI:10.1016/j.trsl.2021.11.009.
[25] Leighton LJ, Chaudhary N, Tompkins HT, et al. The design，manufacture and LNP formulation of mRNA for research use [J]. Nature Protocols，2025，20 (12)：3552-3581. DOI:10.1038/s41596-025-01174-4.
[26] Fiolet T, Kherabi Y, MacDonald CJ, et al. Comparing COVID-19 vaccines for their characteristics，efficacy and effectiveness [J]. Clinical Microbiology and Infection, 2022, 28 (2): 202-221. DOI:10.1016/j.cmi.2021.10.005.
[27] Lu J, Wei W, He W. Regulatory perspective for quality evaluation of lipid nanoparticle-based mRNA vaccines in China [J]. Biologicals，2023，84：101700. DOI:10.1016/j.biologicals.2023.101700.
[28] Camperi J, Chatla K, Freund E, et al. Current Analytical Strategies for mRNA-Based Therapeutics [J]. Molecules，2025，30 (7)：1629. DOI:10.3390/molecules30071629.
[29] Sun W, Tong L, Yang Y L, et al. Analysis on Key Points of Finished Product Quality Control of mRNA Vaccines for Infectious Disease Prevention[J]. Chinese Journal of Pharmaceutical Analysis, 2024, 44(5): 912-915. DOI:10.16155/j.0254-1793.2024.05.20.
[30] Han B, Cao M, Cheng Y. Reflections on the WHO-released Guidelines for mRNA Vaccines and Its Enlightenments to China[J]. Shanghai Medical and Pharmaceutical Journal, 2023, 44(9): 67-70+75.