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DEVELOPMENT AND STANDARDIZATION STRATEGY FOR QUALITY CONTROL OF A RECOMBINANT CAPRIPOXVIRUS-VECTORED VACCINE AGAINST BRUCELLOSIS

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ABSTRACT

This work presents the development and implementation of a quality control and standardization strategy for a recombinant capripoxvirus-vectored vaccine against brucellosis. We conducted a comprehensive analysis of World Health Organization guidelines, the State Pharmacopoeia of the Republic of Kazakhstan, and regulatory documents governing quality control of biological medicinal products. Based on this analysis, we established a comprehensive panel of quality parameters encompassing physicochemical, molecular-genetic, biological, and microbiological characteristics of the vaccine, as well as assessment of residual process-related impurities. Using the developed strategy, we evaluated three pilot batches of the recombinant capripoxvirus-vectored brucellosis vaccine.

The results demonstrated that all pilot batches met the established quality and safety requirements. The developed quality control and standardization strategy provides a foundation for further refinement of the evaluation system for recombinant vector-based brucellosis vaccines and for the preparation of regulatory documentation to support their clinical application.

Keywords: recombinant vaccine, quality control, standardization, brucellosis, capripoxvirus.

INTRODUCTION

Brucellosis is a major bacterial zoonosis that continues to adversely affect livestock production and create substantial risks to human health worldwide [1-4]. The zoonotic nature of the disease, the widespread distribution of natural foci, and the substantial economic losses resulting from reduced animal productivity, reproductive disorders, and trade restrictions necessitate the development of effective and safe specific prophylactic measures [5]. Despite decades of use of conventional live vaccines (*B. abortus* S19, RB51, and *B. melitensis* Rev.1), their application is associated with several limitations, including residual virulence, the potential for vaccine strain persistence in vaccinated animals, the production of antibodies that interfere with serological diagnosis, and potential occupational hazards during vaccine manufacturing and administration. These constraints have prompted renewed interest in next-generation recombinant vaccines that combine high safety standards with potent immunogenic properties [6-8].

Viral vector platforms, particularly those based on capripoxviruses, represent a promising direction in modern vaccine development because of their genetic stability, capacity to accommodate relatively large heterologous sequences, and ability to stimulate both antibody-mediated and cell-mediated immunity. Recombinant capripoxvirus constructs engineered to produce immunologically relevant *Brucella* proteins, such as Omp16 and Omp19, may provide an effective platform for the development of safer vaccines against brucellosis [9-11].

In recent years, Kazakhstan has actively advanced research in the development of innovative brucellosis prophylactic measures using molecular biology, genetic engineering, and viral vector technologies. The Institute for Biological Safety Problems has assumed a leading role in these efforts, implementing comprehensive programs for the development

of domestic recombinant vaccines, diagnostic preparations, and quality control methods [12-14].

The implementation of recombinant vaccines in practical settings requires the development of a scientifically sound quality control and standardization strategy that ensures verification of their safety, efficacy, and reproducibility of the manufacturing process [15, 16]. Unlike conventional biological preparations, the quality control system for recombinant vaccines must encompass not only assessment of physicochemical and microbiological characteristics of the finished product, but also confirmation of genetic stability of the construct, integrity of the integrated expression cassette, functional activity of the viral vector, and control of residual process-related impurities and potential contaminants. Therefore, molecular-genetic, virological, microbiological, and biochemical analytical methods are essential components of modern standardization and quality assurance systems for recombinant immunobiological preparations [1, 2, 16, 17].

Standardization of prophylactic preparations comprises a set of measures aimed at establishing quality criteria, control methods, and acceptable regulatory standards throughout all stages of vaccine manufacturing. The absence of unified international and national requirements for quality control of recombinant veterinary vaccines necessitates the development of an internal standardization strategy that accounts for the specific characteristics of the platform used, the manufacturing process, and the biological properties of the preparation [15, 18]. Such a strategy ensures objective assessment of manufacturing process stability, batch reproducibility, and compliance of the finished product with established quality and safety requirements [19].

Current regulatory documents define general principles for quality control of biological medicinal products; however, they do not address platform-specific requirements for recom-

binant capripoxvirus-vectored vaccines expressing *Brucella* antigens. Therefore, developing a comprehensive approach to quality assessment of such vaccines represents an important scientific and practical objective.

The aim of this study was to develop a quality control and standardization strategy for a recombinant capripoxvirus-vectored brucellosis vaccine, and to conduct a comprehensive evaluation of key quality parameters using modern physicochemical, molecular-genetic, virological, and microbiological control methods. Particular emphasis was placed on justifying quality assessment criteria, confirming the genetic authenticity of the viral vector, determining its biological activity, and analyzing parameters characterizing vaccine safety and purity.

The principal contribution of this study is the establishment of an integrated quality control framework specifically tailored to a recombinant capripoxvirus-vectored vaccine against brucellosis. Rather than evaluating individual product characteristics separately, the proposed framework combines confirmation of vector identity, determination of biological activity, assessment of microbiological safety, monitoring of process-related impurities, and physicochemical characterization within a unified standardization system. It also addresses the current lack of platform-specific guidance for recombinant capripoxvirus vaccines and may provide a scientific basis for batch-release testing, validation of analytical procedures, and subsequent regulatory evaluation.

1. MATERIALS AND METHODS

1.1 Materials

Vaccine

Three experimental batches (No. 1, No. 2, and No. 3) of a recombinant capripoxvirus-vectored vaccine against brucellosis expressing *Brucella* spp. antigens were used as the subject of investigation. The vaccine was developed and manufactured at the Institute for Biological Safety Problems (IBSP, Kazakhstan). For testing, samples from three vials of each batch were collected and combined into a single composite sample by mixing equal volumes of the preparation.

Cell Cultures

Primary lamb testis cell cultures prepared in the Cell Biotechnology Laboratory of IBSP were used in the study.

Vero cells were used as the production cell substrate for propagating the recombinant virus during vaccine manufacture. Primary lamb testis (LT) cell cultures were used as indicator cells to determine the infectious activity of the vaccine.

Bacterial Growth Media

Casein Soybean Digest Agar (TSA) was used to determine the total number of viable aerobic microorganisms and to assess bacterial contamination and compliance with established microbiological purity requirements.

Sabouraud Dextrose Agar (SDA) was used to monitor fungal contamination and to identify and enumerate yeasts and molds.

Test Kits, Assay Systems, and Reagents

InnuPREP DNA/RNA Mini Kit (IST Innuscreen GmbH, Berlin, Germany) - kit for genomic DNA extraction from tissue samples

Taq DNA Polymerase (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) -kit for detection of *brucella* gene inserts in the recombinant capripoxvirus preparation

LAL Reagent ENDOSAFE (Charles River Laboratories, Charleston, SC, USA) - was used for the determination of bacterial endotoxins by the gel-clot LAL method

PerfectStart® Vero DNA Quantification Kit (TransGen Biotech Co., Ltd., Beijing, China) - kit for quantification of residual Vero cell DNA

EZ-PCR Mycoplasma Detection Kit (Sartorius Biological Industries, Israel) - kit for mycoplasma detection

1.2 Methods

Assessment of Appearance

Assessment of appearance is a mandatory component of quality control for biological preparations and serves to confirm compliance with established physicochemical standards, as well as to identify signs of contamination, manufacturing defects, or improper storage conditions.

Appearance was assessed by visual inspection of vials against transmitted light. Clarity, color, and homogeneity of the preparation were evaluated, and the presence of particulate matter, precipitate, floccules, signs of stratification, and mold growth were assessed. Additionally, the integrity of the vials, seal quality, and correctness of labeling were verified. (Figure 1).

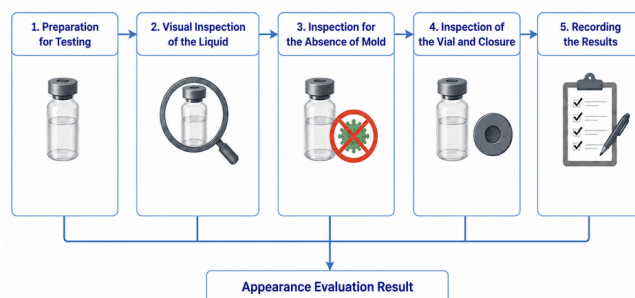


Figure 1. Assessment protocol for visual inspection of a capripoxvirus-vectored brucellosis vaccine.

Hydrogen Ion Concentration (pH)

Hydrogen ion concentration (pH) was measured using a pH meter. The method is based on measuring the potential difference between two electrodes of the pH meter immersed in the test sample [20].

DNA Extraction

Genomic DNA was extracted from samples using the InnuPREP DNA/RNA Mini Kit according to the manufacturer's instructions. The extracted DNA was eluted in 100 µL buffer and stored at -20°C.

Authenticity Testing

The authenticity of the vaccine batches was confirmed using polymerase chain reaction (PCR). Nucleic acid extraction was performed using the InnuPREP DNA/RNA Mini Kit, and amplification was carried out using Taq DNA Polymerase in accordance with the manufacturer's instructions.

To confirm integration of the insert into the thymidine kinase (*TK*) gene locus, primers PCR-TK-F and PCR-TK-R1, flanking the recombination region, were used. The PCR prod-

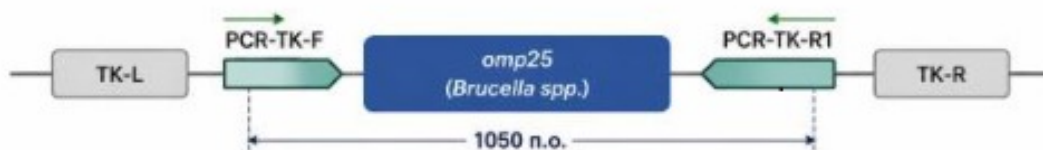


Figure 2. Schematic representation of the *omp25* gene insertion site and PCR primer locations in the recombinant capripoxvirus vector.

uct size for the parent virus strain was 400 bp, whereas for the recombinant strain rSPPV(TK)-*Omp25*, the expected amplicon size was 1050 bp (Figure 2). The amplified fragments were separated in a 1.5% agarose gel prepared with TAE buffer and stained with ethidium bromide. DNA bands were visualized using a GelDoc Go imaging system (Bio-Rad) with a 5-megapixel camera [20].

Determination of Infectious Activity

The infectious activity of each vaccine batch was evaluated in vitro by titration in primary lamb testis (LT) cell cultures prepared at the Cell Biotechnology Laboratory of the Research Institute for Biological Safety Problems (RIBSP). Eight successive tenfold dilutions, ranging from 10^{-1} to 10^{-8} , were prepared in maintenance medium. The first dilution was obtained by combining 0.1 mL of the vaccine preparation with 0.9 mL of medium. For each subsequent dilution, 0.1 mL was transferred into a new tube containing 0.9 mL of fresh medium, with a new pipette tip used at every step.

Four replicate cell culture tubes were inoculated with 0.2 mL of each dilution. Four additional tubes containing non-inoculated cells and maintenance medium served as negative controls. The cultures were maintained at $37 \pm 0.5^\circ\text{C}$, and the medium was replaced every 2 days.

On day 12, the cultures were examined for the development of a cytopathic effect. The assay was considered valid when characteristic cytopathic changes were observed in inoculated cultures and remained absent in the negative controls. The infectious virus titer was calculated by the Reed–Muench method and expressed as $\lg \text{TCID}_{50}/\text{mL}$ [20].

Assessment of Microbiological Purity

Microbiological purity was determined under aseptic conditions. Prepared sample (minimum 0.1 mL) was inoculated onto the surface of pre-dried nutrient medium in Petri dishes and spread evenly using a sterile spreader. Each dilution was examined in duplicate on each type of culture medium.

Casein soybean digest agar plates were maintained at $30\text{--}35^\circ\text{C}$ for 3–5 days to detect aerobic microorganisms. Sabouraud dextrose agar plates were maintained at $20\text{--}25^\circ\text{C}$ for 5–7 days to detect yeasts and molds.

Following incubation, microbial growth was quantified by counting colony-forming units (CFU). Only plates containing no more than 250 colonies for aerobic microorganisms and no more than 50 colonies for yeasts and molds were included in the calculations [20].

Determination of Bacterial Endotoxin Content

Bacterial endotoxins were determined using the gel-clot Limulus amoebocyte lysate (LAL) method with ENDOSAFE® LAL Reagent. Equal volumes of the LAL reagent and test sample were mixed and incubated at 37°C for 60 min. Following incubation, the tubes were inverted by 180° . Forma-

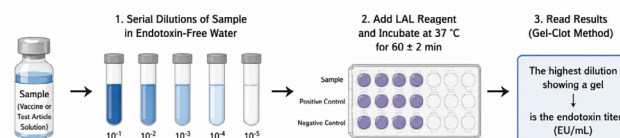


Figure 3. Bacterial endotoxin determination by LAL gel-clot assay.

tion of a firm gel that remained intact upon inversion was considered a positive reaction [20] (Figure 3).

Determination of Residual Benzonase Content

Residual Benzonase content was determined by enzyme-linked immunosorbent assay (ELISA) using a calibration curve constructed from standard solutions with concentrations ranging from 0 to 1000 pg/mL. Optical density was measured at wavelengths of 450 and 630 nm, and results were calculated as the difference $\text{OD}_{450} - \text{OD}_{630}$. For each standard, the mean optical density was determined from three parallel measurements, ensuring improved accuracy and reliability of the data. The calibration curve was subsequently used to determine residual Benzonase content in test samples [18, 20].

Each standard and each test sample was analyzed in three independent parallel replicates ($n = 3$). For each standard, the mean corrected optical density was calculated and used to construct the calibration curve. Experimental data were fitted using a 4-parameter logistic (4-PL) model, which optimally describes the nonlinear kinetics of immunochemical reactions. The constructed calibration model demonstrated a high degree of fit to the experimental data and was sub-

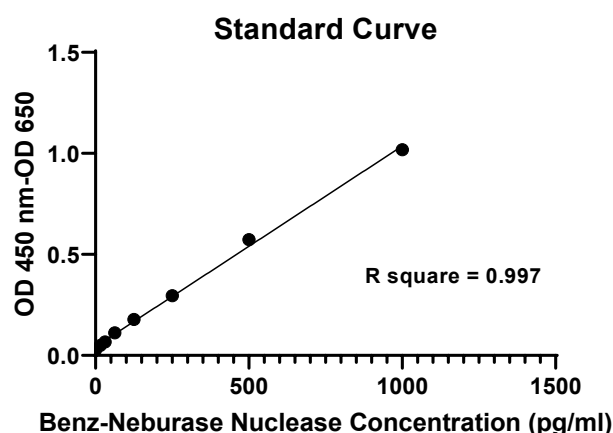


Figure 4. Standard calibration curve for Benzonase Nuclease concentration determination (pg/mL) constructed using 4-parameter logistic regression.

Table 1. Quality control requirements for a recombinant capripoxvirus-vectored brucellosis vaccine

Quality Control Parameter	Test Method	Acceptance Criteria
Appearance	Visual inspection, State Pharmacopoeia of RK Vol. I	Clear or slightly opalescent, colorless liquid, free from particulate matter
Hydrogen ion concentration (pH)	Potentiometric, State Pharmacopoeia of RK Vol. I	6.8-7.6
Authenticity	Polymerase chain reaction (PCR)	Presence of two PCR products of 1050 and 1500 bp
Specific activity	Titration in lamb testis cell culture with calculation of Ig TCID _{50/mL}	Not less than 10 ⁵ TCID _{50/mL}
Microbiological purity	Culture on Casein Soybean Digest Agar (TSA) for TAMC and Sabouraud Dextrose Agar (SDA) for TYMC, State Pharmacopoeia of RK Vol. I	Total Aerobic Microbial Count (TAMC): not more than 1.0×10^2 CFU/mL; Total Yeast and Mold Count (TYMC): not more than 1.0×10^1 CFU/mL
Mycoplasma detection	Polymerase chain reaction (PCR), State Pharmacopoeia of RK Vol. I	Preparation must be free from mycoplasmas
Related substances (cellular DNA)	Polymerase chain reaction (PCR)	Cellular DNA not more than 10 ng/mL
Bacterial endotoxin content	LAL test (gel-clot method), State Pharmacopoeia of RK Vol. I	Not more than 100 EU/mL
Residual Benzonase	Enzyme-linked immunosorbent assay (ELISA)	Not more than ≤ 100 pg/mL

sequently used for accurate interpolation and calculation of trace amounts of residual nuclease in test samples. The obtained values were compared against the established acceptance criterion, according to which Benzonase content should not exceed 100 pg/mL.

A standard calibration curve was constructed based on the mean absorbance values (Figure 4). The X-axis indicates Benzonase Nuclease concentration (pg/mL) and the Y-axis shows the mean corrected optical density.

Determination of Residual Producer Cell DNA

DNA was extracted from samples using the InnuPREP DNA/RNA Mini Kit according to the manufacturer's instructions. Quantitative determination of residual Vero cell DNA was performed by real-time PCR using the PerfectStart Vero DNA Quantification Kit. Standard dilutions of control Vero DNA and a negative control were used for analysis. Residual DNA content was calculated from the calibration curve and expressed in ng/mL of preparation. Since Vero cells were used as the production cell substrate, residual host-cell DNA was quantified using the PerfectStart Vero DNA Quantification Kit according to the manufacturer's instructions.

Detection of Mycoplasma Contamination

Test samples were screened for mycoplasma contamination by PCR using the EZ-PCR Mycoplasma Detection Kit. The assay was performed in accordance with the protocol supplied by the manufacturer. Conserved mycoplasma genomic sequences were amplified, while an internal amplification control was included to confirm assay validity.

Statistical Analysis

All experiments were conducted in at least three independent replicates unless otherwise specified. Determination of pH, specific activity, residual Benzonase content, and other quantitative parameters were performed in triplicate for each vaccine batch. The obtained results were processed using descriptive statistical methods in Microsoft Excel 2019.

RESULTS

A review of available literature revealed the absence of a unified quality control and standardization scheme for recombinant vector vaccines against brucellosis. While existing regulatory documents and scientific publications contain general requirements for evaluating the quality of immunobiological preparations, they do not provide a comprehensive approach to the control of recombinant vaccines based on capripoxvirus vectors.

To establish the regulatory basis for the proposed framework, we examined the relevant WHO guidance, the State Pharmacopoeia of the Republic of Kazakhstan, and Council Decision No. 89 of the Eurasian Economic Commission dated November 3, 2016, concerning the requirements for studies of biological medicinal products within the Eurasian Economic Union [1, 2, 17, 19, 20]. Collectively, these documents define fundamental requirements related to the quality, safety, and efficacy of biological products.

On the basis of this regulatory and scientific assessment, an integrated standardization and quality control strategy was developed for the recombinant capripoxvirus-vectored brucellosis vaccine. The proposed strategy enables systematic evaluation of the vaccine's critical quality attributes, as summarized in Table 1.

The quality control requirements for a recombinant capripoxvirus-vectored brucellosis vaccine were developed taking into account the specific characteristics of the preparation design, its manufacturing process, and current approaches to standardization of vector vaccines. The developed quality control strategy includes assessment of physicochemical, molecular genetic, biological, and microbiological parameters, as well as determination of residual process-related impurities.

Assessment of appearance and pH allows confirmation of compliance of the preparation with established physicochemical characteristics and stability of its composition. Vaccine authenticity is confirmed by polymerase chain reaction (PCR).

Table 2. Quality parameters of pilot batches of a recombinant capripoxvirus-vectored brucellosis vaccine

Quality Parameter	Test Method	Acceptance Criteria	Pilot Batch Results		
			1	2	3
Appearance	Visual inspection	Homogeneous, clear or slightly opalescent liquid without particulate matter, precipitate, floccules, and signs of contamination	Slightly opalescent, homogeneous, colorless, clear liquid. No particulate matter, foreign matter, precipitate, floccules, signs of stratification, or mold detected.		
Hydrogen ion concentration (pH)	Potentiometric	6.8 – 7.6	7.31 ± 0.0027	7.32 ± 0.0027	7.31 ± 0.0028
Authenticity	Polymerase chain reaction (PCR)	Presence of specific amplicon of <i>omp25</i> gene of 1500 bp and 1050 bp	Positive		
Specific activity	Titration in lamb testis cell culture with calculation of lg TCID ₅₀ /mL	Not less than 5.0 lg TCID ₅₀ /mL	5.08 ± 0.14	5.16 ± 0.14;	5.00 ± 0.00
Microbiological purity	Culture on Casein Soybean Digest Agar (TSA) and Sabouraud Dextrose Agar (SDA)	Absence of microbial growth	Negative		
Mycoplasma detection	Polymerase chain reaction (PCR)	Free from mycoplasmas	Negative		
Related substances (cellular DNA)	Polymerase chain reaction (PCR)	Cellular DNA not more than 10 ng/mL	3	6	3
Bacterial endotoxin content	LAL test (gel-clot method)	Not more than 100 EU/mL	15		
Residual Benzonase	Enzyme-linked immunosorbent assay (ELISA)	Not more than ≤100 pg/mL	18	20	23

During analysis, the test sample should demonstrate two specific PCR products of 1050 and 1500 base pairs, corresponding to the genetic construction of the recombinant capripoxvirus vector. The absence of amplicons of the specified sizes or their non-conformance to expected characteristics is not acceptable. The presence of target amplicons indicates the integrity of the integrated genetic insert and confirms vaccine authenticity. Specific activity is determined by titration in sensitive cell culture with calculation of infectious titer in lg TCID₅₀/mL, characterizing the ability of the viral vector to replicate and ensuring reproducibility of the biological activity of the preparation. Microbiological purity is assessed in accordance with the State Pharmacopoeia of the Republic of Kazakhstan by determining the total count of aerobic mesophilic microorganisms (TAMC) and total count of yeasts and molds (TYMC).

Particular attention is devoted to controlling the biological safety of the preparation. To exclude contamination of cell cultures, mycoplasma detection is performed by PCR. Residual cellular DNA content is determined as an indicator of vaccine purification effectiveness and preparation safety. Additionally, the level of bacterial endotoxins is monitored by LAL test, and residual Benzonase content is determined by enzyme-linked immunosorbent assay, confirming the removal of process-related impurities used in the manufacturing process.

Using the developed quality control requirements for the recombinant capripoxvirus-vectored brucellosis vaccine, quality control of three pilot batches of the finished preparation was conducted (Table 2).

Based on the results of quality parameter assessment for

three pilot batches of a recombinant capripoxvirus-vectored brucellosis vaccine, it was established that all tested batches met the established requirements for physicochemical, biological, and microbiological parameters, demonstrating reproducibility of the manufacturing process and stability of the preparation's principal characteristics.

The vaccine was a slightly opalescent, homogeneous, colorless, clear liquid without particulate matter or signs of contamination. pH values ranged from 7.31 to 7.32, complying with established standards. These parameters characterize the preservation of the physicochemical properties of the preparation, confirm the homogeneity of the tested batches, and indicate the absence of degradation or manufacturing process failure. Vaccine authenticity was confirmed by PCR through detection of specific amplicons of the *omp25* gene of expected size, indicating the integrity of the recombinant genetic construct and confirming the identity of the tested batches.

Specific activity of the tested batches ranged from 5.00 to 5.16 lg TCID₅₀/mL and exceeded the established minimum acceptable level. This parameter is of critical importance for confirming the biological activity of the viral vector and ensuring reproducibility of its functional properties, which determine the ability of the vaccine to induce a specific immune response. During microbiological purity assessment and mycoplasma testing, no signs of contamination were detected, confirming the microbiological safety of the preparation and the effectiveness of maintaining aseptic conditions throughout its manufacturing process.

Residual cellular DNA content ranged from 3 to 6 ng/mL and did not exceed the acceptable value. The level of bacterial endotoxins was within the established requirements. Re-

sidual Benzonase concentration was 18 - 23 pg/mL, which was significantly below the maximum allowable level. These results demonstrate the effectiveness of vaccine purification processes, minimization of residual process-related impurities, and compliance of the preparation with current safety requirements for recombinant immunobiological preparations.

DISCUSSION

The obtained results confirm that the developed quality control and standardization strategy for a recombinant capripoxvirus-vectored brucellosis vaccine enables comprehensive assessment of the key parameters that determine quality, safety, and reproducibility of the biopreparation. Unlike conventional live brucellosis vaccines, recombinant vector vaccines require not only confirmation of infectious activity and microbiological purity, but also control of genetic construct integrity, authenticity of the integrated antigen gene, absence of extraneous contamination, residual process-related impurities, and vector stability [17, 22].

According to published studies [15, 16, 23, 24], the quality evaluation of recombinant viral-vector vaccines should include confirmation of product identity, determination of biological activity, sterility testing, verification of freedom from mycoplasma contamination, assessment of residual host-cell DNA, and stability studies. These control parameters are consistent with the principles outlined in relevant European regulatory guidance for live recombinant viral-vectored vaccines and in WHO recommendations concerning recombinant biological products and the cell substrates used in their manufacture. Collectively, these documents emphasize the need to verify the identity of the recombinant vector, demonstrate the genetic stability of the inserted expression cassette, determine biological activity, confirm microbiological purity and the absence of adventitious agents, and monitor residual substances associated with the production process. Therefore, the system of parameters chosen in this work corresponds to current international principles of recombinant biopreparation quality control.

It should be noted that the proposed quality control strategy does not replace existing international regulatory approaches, but rather develops them with respect to the recombinant capripoxvirus platform. EDQM guidelines on official control of batches of immunological veterinary preparations provide general requirements for confirming authenticity, safety, purity, and biological activity of vaccines, as well as product-specific control schemes for live bacterial and certain live viral vaccines. However, these documents do not contain specialized recommendations for recombinant capripoxvirus vector vaccines expressing heterologous antigens. Therefore, the proposed system of tests combines established quality parameters with additional criteria reflecting the characteristics of the viral vector platform, including confirmation of the integrity of the integrated *omp25* gene, assessment of residual process-related impurities, and control of the characteristics of the recombinant viral vector [17, 19, 22, 23].

Of particular importance in the present study was the confirmation of the identity of the recombinant capripoxvirus vector by PCR. The *omp25* gene encodes a conserved outer membrane protein of *Brucella spp.* and belongs to a group of immunologically relevant outer membrane proteins that also

includes *Omp16* and *Omp19*. According to published studies, *Omp16*, *Omp19*, and *Omp25* are considered promising antigenic targets for the development of recombinant vaccines against brucellosis. In the present study, the *omp25* sequence was used as a molecular target to demonstrate a PCR-based approach for confirming the identity of the recombinant capripoxvirus vector construct. Detection of a specific amplicon of the expected size confirmed the integrity and presence of the target genetic insert within the recombinant construct [9, 12].

Comparison with previously published work on influenza vector vaccines against brucellosis shows that most research has been primarily focused on evaluating the immunogenicity, protective activity, and safety of candidate vaccines. Recombinant influenza viruses expressing *Brucella* L7/L12 and *Omp16* antigens were considered as a promising platform, and subsequent studies demonstrated the feasibility of using an influenza vector vaccine against *Brucella abortus* in Kazakhstan's veterinary practice. The present study is focused not on evaluating immunological efficacy, but on developing a system of standardization and quality control for vaccine batches, which is a necessary step for proceeding to comprehensive preclinical and manufacturing studies [22].

The infectious titers of the vaccine batches ranged from 5.00 to 5.16 lg TCID₅₀/mL, confirming the biological activity of the recombinant capripoxvirus vector. For live viral-vector vaccines, infectious titer represents an important quality attribute associated with dose consistency and potential immunogenicity. Khantimirova et al. emphasized that the quality assessment of live recombinant viral-vector vaccines should include comprehensive characterization of the vector and transgene, together with evaluation of the genetic and phenotypic stability of the recombinant virus [23].

An important distinguishing feature of this work is the use of a capripoxvirus vector. This platform is of interest due to its ability to stably carry foreign genes and provide antigen expression within a live viral vector. Current research shows that recombinant capripoxviruses expressing *Brucella* antigens can efficiently replicate in *Vero* cell culture, and the use of bio-reactor systems allows increasing cell density and scaling up the cultivation process [25]. In this respect, the results of this work complement the existing data, as they demonstrate not only the feasibility of producing a recombinant capripoxvirus preparation, but also approaches for its batch-wise quality control based on critical quality parameters.

Physicochemical parameters of the tested batches were characterized by high uniformity. The appearance of the vaccine complied with the requirements for liquid biopreparations: the preparation was a slightly opalescent, homogeneous, colorless, clear liquid free from particulate matter and signs of contamination. pH values were within a narrow range of 7.31-7.32, indicating the stability of composition and reproducibility of the manufacturing process [9, 12].

Microbiological control results showed the absence of bacterial, fungal, and mycoplasma contamination in all tested batches. This is of paramount importance because viral vaccine production in cell culture carries the risk of contamination of the cell substrate and culture media by extraneous agents. In WHO guidelines for vaccine and viral vector quality control, microbiological purity, absence of mycoplasmas, and cell substrate control are considered mandatory elements

of the quality assurance system [17, 19].

Assessment of bacterial endotoxin content, residual cellular DNA, and residual Benzonase is also an important component of the proposed control strategy. These parameters allow characterization of the degree of preparation purification and the safety of the manufacturing process.

Thus, a comparative analysis shows that previously published research on recombinant brucellosis vaccines largely confirms the promise of using viral vectors and *Brucella* spp. antigens. However, issues of standardization, batch-wise quality control, and the development of a comprehensive set of quality parameters have been addressed in less detail. The present work fills this gap by offering a comprehensive quality control strategy for a recombinant capripoxvirus vaccine comprising physicochemical, molecular-genetic, virological, microbiological, and biochemical methods.

It should be noted that this study was primarily aimed at developing and practically validating a quality control strategy for a recombinant capripoxvirus-vectored brucellosis vaccine. The obtained results provide a foundation for further refinement of the vaccine standardization system.

CONCLUSION

The obtained results demonstrate the applicability of the developed quality control strategy for assessing pilot batches of a recombinant capripoxvirus-vectored brucellosis vaccine. The conducted studies demonstrated that the tested batches met the main quality parameters as evaluated in the analytical testing performed. The developed approach can serve as a foundation for further standardization of the preparation, validation of analytical methods, conducting stability studies, and preparation of regulatory documentation required for subsequent stages of preclinical development and manufacturing organization.

CONFLICT OF INTEREST

All authors have read and reviewed the content of this article and have no conflicts of interest.

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AUTHOR CONTRIBUTIONS

Conceptualization, K.K. Jekebekov; methodology, S.U. Moldagulova; formal analysis, A.S. Zhakypbek and S.O. Sadikaliyeva; investigation, S.U. Moldagulova, A. Ulankyz, Zh.S. Absatova and E.Zh. Kalimolda; experimental work, A.S. Zhakypbek, Ye.A. Shayakhmetov, E.P. Voronina and A.D. Omurtay; data curation, G.D. Nakhanova, Ye.A. Shayakhmetov, E.A. Bulatov and Zh.T. Amanova; writing-original draft preparation, K.K. Jekebekov; writing-review and editing, E.A. Bulatov; visualization, T.I. Baiseit; supervision, K.A. Shorayeva and S.Sh. Nurabayev; resources, K.A. Shorayeva; funding acquisition, O.V. Chervyakova. All authors have read and agreed to the published version of the manuscript.

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REFERENCES

1. World Health Organization. Brucellosis. Geneva: World Health Organization. Available from: <https://www.who.int/news-room/fact-sheets/detail/brucellosis> (accessed 25 Apr 2026).
2. World Organisation for Animal Health (WOAH). Brucellosis. Paris: WOAH. Available from: <https://www.woah.org/en/disease/brucellosis/> (accessed 25 Apr 2026).
3. Qureshi KA, Parvez A, Fahmy NA, Abdel Hady BH, Kumar S, Ganguly A, et al. Brucellosis: epidemiology, pathogenesis, diagnosis and treatment – a comprehensive review. *Ann Med*. 2023;55 (2):2295398. doi:10.1080/07853890.2023.2295398.
4. Ghanbari M.K., Gorji H.A., Behzadifar M. et al. One health approach to tackle brucellosis: a systematic review // *Tropical Medicine and Health*. – 2020. – Vol. 48. – Art. 86. – DOI: 10.1186/s41182-020-00272-1.
5. Pappas G., Papadimitriou P., Akritidis N., Christou L., Tsianos E.V. The new global map of human brucellosis // *The Lancet Infectious Diseases*. – 2006. – Vol. 6, No. 2. – P. 91–99. – DOI: 10.1016/S1473-3099(06)70382-6.
6. Dorneles E.M.S., Sriranganathan N., Lage A.P. Recent advances in *Brucella abortus* vaccines // *Veterinary Research*. – 2015. – Vol. 46. – Art. 76. – DOI: 10.1186/s13567-015-0199-7.
7. World Organisation for Animal Health (WOAH). Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Chapter 3.1.4. Brucellosis (*Brucella abortus*, *B. melitensis* and *B. suis*) [Электронный ресурс]. – Paris : WOAH. – URL: <https://www.woah.org/en/what-we-do/standards/codes-and-manuals/terrestrial-manual-online-access/>
8. Avila-Calderón E.D., Lopez-Merino A., Sriranganathan N., Boyle S.M., Contreras-Rodríguez A. A history of the development of *Brucella* vaccines // *BioMed Research International*. – 2013. – Vol. 2013. – Art. 743509. – DOI: 10.1155/2013/743509.
9. Pasquevich K.A., Estein S.M., Samartino C.G. et al. Immunological basis of *Brucella* infection and vaccination // *Frontiers in Cellular and Infection Microbiology*. – 2021. – Vol. 11. – Art. 691953. – DOI: 10.3389/fcimb.2021.691953.
10. Gupta V.K., Kumar M., Vohra V. et al. Outer membrane proteins of *Brucella* as vaccine candidates: current status and future prospects // *Microbial Pathogenesis*. – 2022. – Vol. 166. – Art. 105505. – DOI: 10.1016/j.micpath.2022.105505.
11. Logunov DYu, Dolzhikova IV, Shmarov MM, et al. Recombinant viral vectors as a platform for vaccine development. *Biopreparaty. Profilaktika, Diagnostika, Lechenie (Biopreparations. Prevention, Diagnosis, Treatment)*. 2016;16(1). (In Russ.).

12. Tabynov K., Sansyzbay A., Kydyrbayev Z., Yespembetov B., Ryskeldinova S., Zinina N., Assanzhanova N., Sultankulova K., Sandybayev N., Khairullin B., Kuznetsova I., Ferko B., Egorov A. Influenza viral vectors expressing the *Brucella* OMP16 or L7/L12 proteins as vaccines against *B. abortus* infection // *Virology Journal*. – 2014. – Vol. 11. – Art. 69. – DOI: 10.1186/1743-422X-11-69.
13. Tailakova E.T., Sadikaliyeva S.O., Shynybekova G.O., Abubakirova A.K., Sultankulova K.T., Chervyakova O.V. Construction, Expression and Purification of *Brucella* spp. Recombinant Proteins L7/L12 and SodC in *Escherichia coli* // *News of the National Academy of Sciences of the Republic of Kazakhstan. Series of Biological and Medical*. – 2020. – Vol. 2 (338). – P. 20–30. – DOI: 10.32014/2020.2519-1629.9.
14. Assanzhanova N., Zhugunissov K., Chervyakova O., Ryskeldinova S., Akmyrzayev N., Sagymbayeva A., Myrzakhmetov Y., Mailybayeva A. Modern Strategies for Brucellosis Vaccination: From Traditional Approaches to Innovative Platforms // *Vaccines*. – 2026. – Vol. 14, No. 5. – Art. 409. – DOI: 10.3390/vaccines14050409.
15. Tailakova ET, Sultankulova KT, Kasenov MM, Zhunushov AT, Chervyakova OV. Development of a quality control and standardization strategy for a recombinant sheep pox virus protein-based vaccine. *Izvestiya Natsionalnoi akademii nauk Kyrgyzskoi Respubliki*. 2019;(5):56–62. (In Russ.).
16. Eremets V.I., Samuylenko A.Ya., Eremets N.K. i Dr. Sovershenstvovanie i standartizatsiya tekhnologicheskikh protsessov, metodov kontrolya i upravleniya kachestvom protivovirusnykh vaksyn // *Veterinarnyi vrach*. 2011;(3):4–7.
17. World Health Organization. Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks. WHO Technical Report Series No. 978, Annex 3. Geneva: World Health Organization; 2013. Available from: <https://www.who.int/publications/item/WHO-TRS-978-annex-3> (accessed 25 Jun 2026).
18. Kukharenko AE, Gavrilova NA, Gravel IV, Cherepushkin SA, Orlova NV, Lovtsova LV. Standardization of finished dosage form of therapeutic vaccine against human papillomavirus-associated diseases. *Fundamentalnye issledovaniya (Fundamental Research)*. 2014;(12, Pt. 7):1477–1481. (In Russ.).
19. European Directorate for the Quality of Medicines & HealthCare (EDQM). *European Pharmacopoeia*. 11th ed. Strasbourg: EDQM; 2023. Available from: <https://www.edqm.eu/en/european-pharmacopoeia> (accessed 15 Jun 2026).
20. Gosudarstvennaya farmakopeya Respubliki Kazakhstan. Vol. 1. Almaty: Izdatel'skii dom «Zhibek zholy»; 2008. 592 p. (In Russ.).
21. Microsoft Corporation. *Microsoft Excel*. Available from: <https://www.microsoft.com/microsoft-365/excel> (accessed 15 Jun 2026).
22. Decision of the Council of the Eurasian Economic Commission No. 89 of 3 November 2016 «On Approval of the Rules for Conducting Studies of Biological Medicinal Products of the Eurasian Economic Union.» Available from: <https://docs.eaeunion.org/> (accessed 25 Jun 2026).
23. Khantimirova LM, Gorenkov DV, Guseva SG, Aliper TI, Merkulov VA. Approaches to quality control, preclinical and clinical studies of live recombinant viral vector vaccines. *Biopreparaty. Profilaktika, Diagnostika, Lechenie (Biopreparations. Prevention, Diagnosis, Treatment)*. 2021;21(4):212–224. doi:10.30895/2221-996X-2021-21-4-212-224. (In Russ.).
24. Shmidt A.A., Egorova T.V. PCR-Based Analytical Methods for Quantification and Quality Control of Recombinant Adeno-Associated Viral Vector Preparations // *Pharmaceuticals*. – 2022. – Vol. 15, No. 1. – Art. 23. – DOI: 10.3390/ph15010023.
25. Boshra H., Truong T., Nfon C. Capripoxvirus vaccines: recent advances and future perspectives // *Expert Review of Vaccines*. – 2015. – Vol. 14, No. 5. – P. 663–681. – DOI: 10.1586/14760584.2015.1025051.

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РАЗРАБОТКА СТРАТЕГИИ КОНТРОЛЯ КАЧЕСТВА И СТАНДАРТИЗАЦИИ РЕКОМБИНАНТНОЙ ВАКЦИНЫ ПРОТИВ БРУЦЕЛЛЕЗА НА ОСНОВЕ КАПРИПОКСВИРУСНОГО ВЕКТОРА

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АННОТАЦИЯ

В работе представлены результаты разработки стратегии контроля качества и стандартизации рекомбинантной вакцины против бруцеллеза на основе каприпоксвирусного вектора. Проведен анализ международных рекомендаций Всемирной организации здравоохранения, требований Государственной фармакопеи Республики Казахстан и нормативных документов, регламентирующих контроль качества биологических лекарственных средств. На основании проведенного анализа сформирован комплекс показателей качества, включающий оценку физико-химических, молекулярно-генетических, биологических, микробиологических характеристик вакцины, а также содержания остаточных технологических примесей. С использованием разработанной стратегии проведена оценка трех опытных серий рекомбинантной вакцины против бруцеллеза на основе каприпоксвирусного вектора.

Полученные результаты свидетельствуют о соответствии опытных серий вакцины установленным требованиям качества и безопасности. Разработанная стратегия контроля качества и стандартизации может быть использована в качестве основы для дальнейшего совершенствования системы оценки рекомбинантных векторных вакцин против бруцеллеза и подготовки нормативной документации для их практического применения.

Ключевые слова: рекомбинантная вакцина, контроль качества, стандартизация, бруцеллез, каприпоксвирус.

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КАПРИПОКСВИРУСТЫҚ ВЕКТОР НЕГІЗІНДЕГІ БРУЦЕЛЛЕЗГЕ ҚАРСЫ РЕКОМБИНАНТТЫ ВАКЦИНАНЫҢ САПАСЫН БАҚЫЛАУ МЕН СТАНДАРТТАУ СТРАТЕГИЯСЫН ӘЗІРЛЕУ

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АННОТАЦИЯ

Бұл жұмыста каприпоксвирустық вектор негізіндегі бруцеллезге қарсы рекомбинантты вакцинаның сапасын бақылау және стандарттау стратегиясын әзірлеу нәтижелері ұсынылған. Дүниежүзілік денсаулық сақтау ұйымының (ДДСҰ) халықаралық ұсынымдарына, Қазақстан Республикасы Мемлекеттік фармакопеясының талаптарына және биологиялық дәрілік препараттардың сапасын бақылауды регламенттейтін нормативтік құжаттарға талдау жүргізілді. Жүргізілген талдау негізінде вакцинаның физика-химиялық, молекулалық-генетикалық, биологиялық және микробиологиялық сипаттамаларын, сондай-ақ технологиялық процестен қалатын қалдық қоспалардың мөлшерін бағалауды қамтитын сапа көрсеткіштерінің кешені қалыптастырылды.

Әзірленген стратегияны пайдалана отырып, каприпоксвирустық вектор негізіндегі бруцеллезге қарсы рекомбинантты вакцинаның үш тәжірибелік сериясына бақылау жүргізілді.

Алынған нәтижелер вакцинаның тәжірибелік серияларының сапа мен қауіпсіздік бойынша белгіленген талаптарға сәйкес келетіндігін көрсетті. Әзірленген сапаны бақылау және стандарттау стратегиясы бруцеллезге қарсы рекомбинантты векторлық вакциналарды бағалау жүйесін одан әрі жетілдіру және оларды тәжірибелік қолдануға арналған нормативтік құжаттаманы дайындау үшін негіз ретінде пайдаланылуы мүмкін.

Түйінді сөздер: рекомбинантты вакцина, сапаны бақылау, стандарттау, бруцеллез, каприпоксвирус.