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ORIGINAL RESEARCH ARTICLE

Comparative analysis of quality control data for adenovirus serotype 5- and 26-based viral vector vaccine across manufacturing sites

Daria Yu. Kolomiytseva*, Anastasia A. Sirotkina, and Natalia A. Litvinova

Department of Molecular Diagnostics, Cell Testing Laboratory, Generium, Petushinsky District, Vladimir Region, Russia

Abstract

Introduction: The release of pharmaceutical products, including biotechnological viral vector vaccines based on adenovirus serotypes 5 and 26, requires analytical control using suitable methods for both intermediate products and the final drug formulation.

Objective: To demonstrate the stability of the vector vaccine production process against quality criteria using analytical methods and to prove the convergence of data obtained across multiple manufacturing sites.

Methods: Test samples consisted of vaccine batches from three manufacturing sites. Vaccine titer was determined via the 50% cell culture infectious dose method. Authenticity was assessed using an enzyme-linked immunosorbent assay-based biological test. Biosafety was confirmed using the plaque-forming unit assay.

Results: Eighteen samples from three manufacturing sites (six from each) were analyzed in accordance with the specification parameters. The analysis included determination of protein and DNA authenticity, capsid fullness, biosafety, and infectious titer. The authenticity of the DNA insert was also confirmed. The analyzed vaccine batches met the requirements for the content of replication-competent viral particles, proving their biosafety. The average infectious titer of the vaccine ranged from 0.92×10^7 to 4.06×10^7 vp/mL, which complies with the specification.

Conclusion: All tested samples met the established acceptance criteria. The comparability of quality control results across manufacturing sites indicates the reproducibility of the vaccine production process, a key indicator of the stability and reliability of the manufacturing technology.

Keywords: Vaccine preparation; Analytical methods; Quality control; Adenovirus; Coronavirus infection; Data convergence; Authenticity; Biosafety

***Corresponding author:**
Daria Yu. Kolomiytseva
(dykolomiytseva@generium.ru)

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1. Introduction

Vaccination against coronavirus infection (COVID-19) has become one of the key measures in combating the pandemic. The development and deployment of vaccines within a short timeframe required pharmaceutical companies not only to concentrate resources and efforts on development and technology transfer to production but also to ensure rigorous quality control. Vaccine preparations must be safe and effective, necessitating the use of modern analytical methods at all stages of production.[1-5] A similar situation has been noted in many countries, where the emergence of SARS-

CoV-2 significantly altered the epidemiological landscape[6] and prompted a rapid expansion of vaccine development efforts.[7]

The production and certification of recombinant adenoviruses of serotypes 5 and 26 (Ad5, Ad26) as investigational biological products require expertise in complex methods for vector production, purification, and characterization, combined with the application of modern Good Manufacturing Practice (GMP) and comprehensive procedural controls to ensure product purity, authenticity, safety, and consistency.[8-14]

To strengthen the reliability of viral vector production, it is important to ensure that the vaccine maintains the same quality regardless of where it is manufactured.[15,16] When a product is produced at several independent sites, regulators expect clear evidence that its critical attributes do not drift across facilities.[12,17,18] This requirement is directly linked to product reproducibility, patient safety, and the overall robustness of the technology. Consistent results across sites also help exclude the influence of local factors—such as equipment differences, raw material variability, or specific process conditions—which may otherwise introduce unwanted fluctuations in product quality.[12,13,17]

Analytical methods play a central role in vaccine quality control, from controlling intermediates during development to final product quality. They allow verification of vaccine purity and potency, as well as detection of potential impurities or deviations from specified parameters.[1,19-23] Methods such as preparative and analytical ultracentrifugation, spectrophotometry, polymerase chain reaction (PCR), and others are applied at all stages of production.[19,20,21,23]

This study presents a comparative analysis of batches of the "Salnavak®" vector vaccine produced by Generium at several manufacturing sites. The described analytical approaches at various quality control stages enable comprehensive quality monitoring, ensuring the product's efficacy and safety.[17,12,14]

This work analyzes vector vaccine samples against quality criteria using analytical methods and demonstrates the convergence of data obtained across multiple manufacturing sites.[24-26]

2. Materials and methods

The study utilized 18 industrial batches of the combined vector vaccine Salnavak® for the prevention of the novel coronavirus infection caused by SARS-CoV-2 from three different manufacturing sites of JSC Generium. Batch numbers for the first component are T-01, T-02, T-03, T-50, T-57, T-10, TW-01, TW-02, and TW-06. Batch numbers for the second component are F-26, F-27, F-01, F-40, F-64, F-68, F-03, F-06, and F-07.

The vaccine includes two independently manufactured components: Component I, which is based on the Ad26 vector, and Component II, which uses the Ad5 vector.[15,17] Each production site supplied six industrial batches—three for Component I and three for Component II. In total, the study covered 18 batches, providing an equal representation of both components across all manufacturing sites.[27-29]

Six batches were taken from each manufacturing site, reflecting the typical output of routine industrial production. This number corresponds to the volume typically assessed during annual product quality reviews and is sufficient to capture variability within a single site while still allowing meaningful comparisons between sites.

2.1. Cell culture

All cell lines (A-549 [cat No CCL-185; ATCC, United States], HEK293 [cat. No CRL-1573; ATCC, United States]) were cultured adherently in Dulbecco's Modified Eagle Medium (Sigma-Aldrich, United States) containing 10% fetal bovine serum (HyClone, United States) supplemented with glutamine (Sigma-Aldrich, United States) at a concentration of 200 mM and antibiotic penicillin–streptomycin at quantities of 100,000 U/L and 100 mg/L, respectively (LLC Paneko, Russia).[11,19,20]

2.2. Plaque-forming unit assay

The content of replication-competent viral particles in the vaccine preparation was determined using the plaque-forming unit (PFU) assay, based on the virus's ability to cause zones of cytopathic effect (plaques) in a cell monolayer. The A-549 cell line (human lung carcinoma) was used as the cellular substrate.[19-21,23]

Cryovials with cells were thawed in a water bath at (37 ± 1) °C for two min. The contents were transferred to a centrifuge tube with 9 mL of growth medium and centrifuged at 1,000 rpm for 5–7 min. The supernatant was removed, and the pellet was resuspended in 5–10 mL of fresh medium. For viability assessment, 50 μL of the suspension was mixed with an equal volume of 0.4% trypan blue solution, and live cells were counted using an automatic counter.

Cells were cultured in T-75 or T-175 flasks under standard conditions (37 °C**,** 5% carbon dioxide). Passaging was performed every 2–3 days at a seeding density of 1–2 × 10^5 cells/mL, with mandatory monitoring of morphology and confluency by microscopy. The total number of

passages did not exceed 20.[24,26] Cells in the logarithmic growth phase, forming a uniform monolayer, were used for analysis.

Before the experiment, the culture medium was removed, and cells were treated with 3 mL of 0.25% trypsin–ethylenediaminetetraacetic acid solution for washing. Subsequently, 2–3 mL of fresh trypsin–ethylenediaminetetraacetic acid solution was added, and the samples were incubated for 3–5 min until complete cell detachment (monitored by microscopy [Eclipse TS100F, Nicon, Japan]). Cells were resuspended in 6–8 mL of growth medium, transferred to a centrifuge tube, washed to remove trypsin residues (1,000 rpm, 5 min), pooled, and adjusted to a density of 1.5×10^5 cells/mL.[3]

2.3. The 50% cell culture infectious dose method

This method is intended for determining the infectious titer of the vaccine. The analysis was performed by adding serial dilutions of the virus sample to HEK293 cells in a 96-well plate. After incubation, cells were stained with vital dyes based on tetrazolium salts (XTT, MTT), and each well was classified as infected or uninfected. The dilution at which 50% of the wells show a cytopathic effect is used to calculate the 50% cell culture infectious dose ($CCID_{50}$) of the virus sample using the GraphPad Prism software version (10.4.1).[19,20-23] The virus titer was expressed as $CCID_{50}$/mL. HEK293 cells were cultured in Dulbecco's Modified Eagle Medium containing 10% fetal bovine serum. The culture medium was supplemented with glutamine at a concentration of 200 mM and penicillin–streptomycin antibiotic.[11,20,21]

2.4. Enzyme-linked immunosorbent assay

A suspension culture of HEK293 cells was thawed using the standard procedure per the manufacturer's recommendations, and 2–3 passages were performed to adapt the cells. Lysates were obtained by three freeze–thaw cycles. Cell debris was precipitated by centrifugation at 3,700 rpm for 20 min. The supernatant was then transferred to a separate 96-well non-binding plate. The supernatant from the lysates was then used as the antigen in an enzyme-linked immunosorbent assay (ELISA).[19-23]

Calculation was performed automatically using GraphPad Prism (version 10.4.1). A sigmoidal curve indicates the presence of the S protein in the sample, demonstrating the authenticity of the produced product. A straight line parallel to the X-axis indicates the absence of the S protein and no specific activity. The acceptance criterion for this parameter is the detection or non-detection of the S protein.[27,28]

2.5. Polymerase chain reaction

DNA extraction was performed using the ProGen (South Korea) kit according to the manufacturer's instructions. Diethyl pyrocarbonate (DEPC)-treated water was used as a negative control. A positive control sample for the human adenovirus serotype 26 *hexon* gene (pAd26wt) was diluted tenfold with the DNA Rehydration Solution (Promega, United States).[19-21,23]

Polymerase chain reaction was performed using a CFX96 Touch Real-Time PCR Detection System (Bio-Rad, United States). The amplification program used is as follows: (i) polymerase activation: 95 °C for five min; and (ii) 40 cycles of denaturation: 95 °C for 15 s, annealing: 60 °C for 20 s, and elongation: 72 °C for 20 s. DNA concentration was calculated using a calibration curve of standard solutions with DNA quantities per well (ng): 27, 3, 0.3, 0.03, 0.003. Positive control samples used were: (i) positive control sample for human adenovirus serotype 5 *hexon* gene pAdEasy (100 ng/µL); (ii) positive control sample for human adenovirus serotype 26 *hexon* gene pAd25 wt (100 ng/µL); (iii) positive control sample for SARS-CoV-2 S protein gene *pSh26-S-Nov* (100 ng/µL). Hexachlorofluorescein (HEX), fluorescein amidite (FAM), and SYBR reading channels were used for detection.[11,19–22]

2.6. Spectrophotometry

The absorption and scattering spectra in the near-ultraviolet region (260–320 nm) are characteristic of virus-like particles and provide a simple, rapid, and direct method for the quantitative determination of viral particles in solution.[30] For viral particles with a full-length genome, the ratio of optical absorbance at 260 nm to 280 nm ranges from 1.2 to 1.4, whereas for empty capsids the ratio ranges from 0.5 to 0.8. The equipment used for spectrophotometry was a multimode plate reader, SpectraMax M5 (Molecular Devices, United States).

Results were considered valid if the measured optical density value at 260 nm was between 0.1 AU and 1.0 AU. A260 and A280 values were calculated with corrections using the following formulas (**Equations 1**–**3**):

$$\text{Corr A260} = (\text{A260} - \text{A320}) \quad (1)$$

$$\text{Corr A280} = (\text{A280} - \text{A320}) \quad (2)$$

$$\text{Adjusted ratio} = (\text{Corr A260} / \text{Corr A280}) \quad (3)$$

where A260 is the mean optical density value at wavelength 260 nm for three sites, A280 is the mean optical density

value at wavelength 280 nm for three sites, and A320 is the mean optical density value at wavelength 320 nm for three sites. The ratio A260−A320/A280−A320 should be within 1.1–1.5.[22,24]

The particle content in one dose (0.5 mL) of Component I and Component II was determined using **Equation 4**:

$$X = 1.1 \times 10^{12} \times \mathrm{Corr}\,A_{260} \times 0.5 \quad (4)$$

where 1.1×10^{12} is the number of particles in 1 mL at 260 nm, and 0.5 is the dose volume.

2.7. Statistical indicators

Table 1 presents the formulas used to calculate the statistical indicators.

2.8. Statistical analysis

The infectious titer per dose, expressed as CCID50, was statistically compared between manufacturing sites for each vaccine component separately using one-way analysis of variance. Statistical analysis was performed using GraphPad Prism software version 10.4.1. The *F*-statistic was calculated as the ratio of the mean square between groups to the mean square within groups. Sum of squares, mean squares, degrees of freedom, *F*-values, and *p*-values were determined. A significance threshold of $p < 0.05$ was applied. Differences were considered statistically significant when the calculated *p*-value did not exceed the predefined significance level.

3. Results

3.1. Method specifications

A complex of various methods was used in the study, in accordance with regulatory requirements for vaccines and gene therapy products (Table 2).[17,12-14,25,31] ELISA was used to confirm the authenticity of the expressed protein, providing high specificity through antibody detection. The method is recommended for controlling the identity and expression of proteins in recombinant preparations.[24]

Polymerase chain reaction was used to detect nucleic acids encoding coronavirus proteins and to identify capsid sequences of adenoviruses of different serotypes. The method is highly sensitive but cannot distinguish replicating particles from defective ones; therefore, it is not suitable for assessing infectivity but can be used to confirm the authenticity of genetic constructs.[15,16]

The PFU assay detects replication-competent adenoviruses by detecting cytopathic effect. According to the European Medicines Agency and the Food and Drug Administration guidelines, functional cell-based tests like PFU are preferred for assessing the safety of viral vectors.[12,17,18] $CCID_{50}$ was used to assess infectious titer and accounts only for functionally active viral particles.[24]

Spectrophotometry was used as a rapid method for estimating total viral particle count. Despite its low specificity, the method remains in demand at various stages of quality control.[22] The combination of these approaches provides a comprehensive characterization of vector-based drugs and vaccines with respect to authenticity, specific activity, and safety, in accordance with the quality control system for virus-containing preparations.[18,24-26,31]

3.2. Quantitative determination

The obtained results for the quantitative content of vector particles per vaccine dose for Components I and II ranged from 0.9 to 1.1×10^{11} particles/dose.[18,22,23] The obtained values of viral particle concentration are given in Table 3.[18,25] These values indicate the homogeneity of the investigated preparations. This allows us to assess the stability of the production process and the reproducibility of product characteristics.

3.3. Polymerase chain reaction

The authenticity of the expression cassette and the presence of the target insert were confirmed using PCR. This method allows the detection of target nucleotide sequences corresponding to the construct structures.[15,19-22]

Amplification of fragments corresponding to key regions of the cassette confirmed its integrity and the absence of recombination. The obtained results match the expected ones, indicating the stability of the construct and the correctness of the expression system assembly.[16]

Polymerase chain reaction analysis results, detected in the FAM and HEX channels, show confident detection of the adenovirus serotypes 26 and 5 *hexon* genes in all investigated batches, regardless of the manufacturing site (Table 4). Low Ct values in the FAM channel (less than 30) and HEX channel (not more than 20) fully comply with the established authenticity criteria. Positive controls (pAd26wt) and negative controls (DEPC-water) also yielded the expected results, confirming the accuracy of the PCR analysis and the reliability of all data obtained.

Polymerase chain reaction analysis with detection in the SYBR channel confirmed the presence of the SARS-CoV-2 S protein gene in all vaccine batches, regardless of the manufacturing site (Table 5). All samples showed Ct values within the positive result criteria (<20), indicating reliable detection of the target gene. Positive (pSh26-S-

Nov) and negative (DEPC-water) controls also yielded correct results, ensuring the method's high accuracy and confirming the authenticity of the expression construct in the investigated batches.

The obtained data allow assessment of the authenticity of the expression cassette using the selected primers for the genes responsible for coronavirus S protein production. The authenticity of the expression cassette and the construct was confirmed.

3.4. Plaque-forming unit assay

Vaccine samples were analyzed for the specific safety criterion using the PFU assay. The obtained values were interpreted according to the criteria established in a previous study during method validation.[32]

Figure 1 illustrates the appearance of culture flasks with agarized medium and cell monolayers, affected and unaffected by replication-competent adenoviruses. In the virus control flasks, individual plaques— zones of A-549 cell lysis caused by wild-type adenovirus, serotype 5—were observed, confirming the method's functionality.

Table 1. Formulas for calculating statistical indicators

Statistical indicator	Calculation formula
Arithmetic mean	$\overline{X} = \frac{\sum_{i=1}^{n} Xi}{n}$
Standard deviation	$S = \sqrt{\frac{\sum_{i=1}^{n}\left(X_i - \overline{X}\right)^2}{n-1}}$
Coefficient of variation (relative standard deviation)	$RSD = \frac{S}{X_{cp}} \times 100\%$

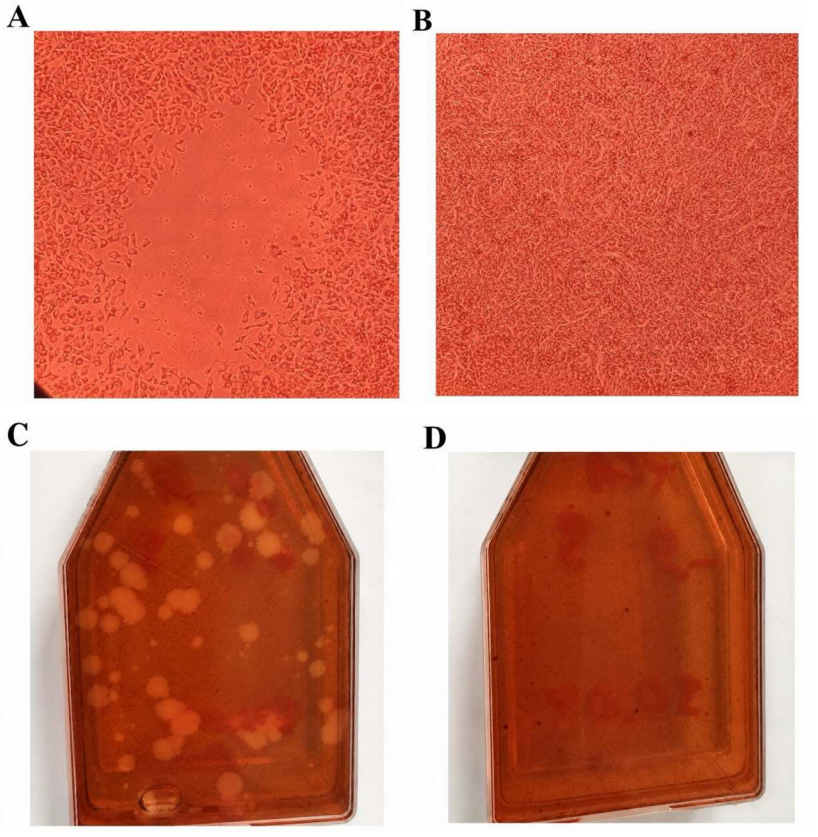

Figure 1. Microscopy of A-549 cell monolayer. (A) After the addition of wild-type adenovirus. (B) Negative control. (C) View of monolayer after addition of wild-type adenovirus. (D) View of monolayer without addition (negative control). Scale bar: N/A; magnification: 150×.

Table 2. Specification for biological methods

Parameter	Method	Acceptance criteria	Conformity
Safety	Cell-based test (Plaque assay on A549 cell culture)	≤3 replication-competent adenoviruses per dose	Conforms
Potency (biological activity)	Infectious titer of recombinant adenovirus using 50% cell culture infective dose ($CCID_{50}$)	The number of active recombinant adenoviral particles must be not less than 0.50×10^7 per dose	Conforms
Quantitative determination	Spectrophotometry	Must contain $(1.0 \pm 0.5) \times 10^{11}$ adenoviral particles/dose	Conforms
Authenticity (identity)	Enzyme-linked immunosorbent assay	Formation of an S-shaped curve	Conforms
	Real-time polymerase chain reaction	Must contain the *hexon* gene of recombinant human adenovirus serotype 26 and the glycoprotein S gene of SARS-CoV-2 virus, and the *hexon* gene of recombinant human adenovirus serotype 5 and the glycoprotein S gene of SARS-CoV-2 virus	Conforms

Table 3. Concentration values of vaccine samples obtained by spectrophotometry

Sample	Particles/mL	Particles/dose
T-01	2.17×10^{11}	1.1×10^{11}
T-02	1.79×10^{11}	0.9×10^{11}
T-03	2.19×10^{11}	1.1×10^{11}
T-50	2.18×10^{11}	1.1×10^{11}
T-57	1.79×10^{11}	0.9×10^{11}
T-10	1.78×10^{11}	0.9×10^{11}
TW-01	1.93×10^{11}	1.0×10^{11}
TW-02	1.88×10^{11}	0.9×10^{11}
TW-06	1.69×10^{11}	0.9×10^{11}
F-26	1.80×10^{11}	0.9×10^{11}
F-27	2.27×10^{11}	1.1×10^{11}
F-01	2.11×10^{11}	1.1×10^{11}
F-40	2.02×10^{11}	1.0×10^{11}
F-64	1.72×10^{11}	0.9×10^{11}
F-68	2.07×10^{11}	1.1×10^{11}
F-03	1.77×10^{11}	0.9×10^{11}
F-06	1.81×10^{11}	0.9×10^{11}
F-07	2.12×10^{11}	1.0×10^{11}

Analysis of the specific safety of all vaccine batches from three manufacturing sites showed that monolayer destruction was not observed in any sample, indicating the absence of PFU and replication-competent viral particles in the vaccine preparations. The state of the monolayer in the culture flasks with control samples corresponded to the virus titer. The monolayer in the flask with the negative control showed no destruction, and no PFU were detected. The monolayer destruction in the control flasks corresponded to the virus titer, confirming the absence of replication-competent viral particles.

3.5. The 50% cell culture infectious dose method

Quantitative determination of the total number of particles does not allow assessment of the infecting ability of vector viral particles. A biological test is required to obtain this titer.[20,22,23] With a total viral particle titer measured by spectrophotometry of 1.0×10^{10}, the infectious titer is approximately 1.0×10^7.[18,22,24]

The $CCID_{50}$ method was used to analyze the infectious titer of vaccine samples containing recombinant vector particles. The results of the analysis of vaccine samples by the $CCID_{50}$ method are given in Table 6.[24,31,33]

The results for the infectious titer of the viral vector vaccine, calculated using the $CCID_{50}$ method, show stable values across all batches, regardless of the manufacturing site. For the first component, the mean virus titer varied from 0.92×10^7 ($\log_{10} = 6.9638$) to 2.01×10^7 vp/mL ($\log_{10} = 7.3032$), and for the second from 1.77×10^7 ($\log_{10} = 7.2479$) to 4.06×10^7 vp/mL ($\log_{10} = 7.6085$).[15,16,18] Low coefficients of variation (less than 4.5%) within batches indicate the robustness of the process, analytical methods, and stability of the obtained data. Between manufacturing sites, the standard deviation values for Components I and II were 2.23% and 1.38%, respectively. This confirms the quality of the vaccine preparation and the reliability of the

Table 4. Test results for the presence of the *hexon* gene of recombinant human adenovirus serotypes 26 and 5

Name	Batch no	Mean Ct (FAM)	Mean Ct (HEX)	Result interpretation	Presence of recombinant adenovirus *hexon* gene
Component I					
Manufacturing site 1	T-01	11.03	11.60	Ct (FAM) < 30, Ct (HEX) ≤ 20	Present
	T-02	10.76	11.92		
	T-03	10.43	12.06		
Manufacturing site 2	T-50	9.12	9.46		Present
	T-57	13.10	10.16		
	T-10	11.96	10.20		
Manufacturing site 3	TW-01	13.05	7.55		Present
	TW-02	13.15	7.48		
	TW-06	10.71	7.87		
Negative extraction control		11.81	N/A	Ct (FAM) < 30, Ct (HEX) > 20	
Positive control (pAd26wt)		N/A	10.04	Ct (FAM) ≥ 30, Ct (HEX) ≤ 20	Acceptable
Negative control (water)		N/A	N/A	Ct (FAM) ≥ 30, Ct (HEX) > 20	
Component II					
Manufacturing site 1	F-26	11.89	10.71	Ct (FAM) < 30, Ct (HEX) ≤ 20	Present
	F-27	11.32	10.58		
	F-01	9.14	10.63		
Manufacturing site 2	F-40	12.79	9.08		Present
	F-64	13.25	10.82		
	F-68	14.21	12.28		
Manufacturing site 3	F-03	14.03	9.18		Present
	F-06	13.40	10.69		
	F-07	10.15	10.12		
Negative extraction control		11.67	N/A	Ct (FAM) < 30, Ct (HEX) > 20	
Positive control (pAdEasy)		N/A	9.87	Ct (FAM) ≥ 30, Ct (HEX) ≤ 20	Acceptable
Negative control (water)		N/A	N/A	Ct (FAM) ≥ 30, Ct (HEX) > 20	

Abbreviations: Ct: Cycle threshold; FAM: Fluorescein amidite; HEX: Hexachlorofluorescein.

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Table 5. Test results for the presence of the SARS-CoV-2 glycoprotein S gene

Name	Batch no	Mean cycle threshold (Ct)	Result interpretation	Presence of the SARS-CoV-2 protein S gene
Component I				
Manufacturing site 1	T-01	9.03	Ct ≤ 20	Present
	T-02	8.95		
	T-03	8.94		
Manufacturing site 2	T-50	7.61		
	T-57	7.71		
	T-10	7.75		
Manufacturing site 3	TW-01	5.27		
	TW-02	5.05		
	TW-06	5.42		
Negative extraction control (PCR-RV)		24.38	Ct > 20	Acceptable
Positive control (pSh26-S-Nov)		9.81	Ct ≤ 20	
Negative control (water)		24.44	Ct > 20	
Component II				
Manufacturing site 1	F-26	8.88	Ct ≤ 20	Present
	F-27	8.99		
	F-01	9.01		
Manufacturing site 2	F-40	7.82		
	F-64	7.52		
	F-68	7.55		
Manufacturing site 3	F-03	7.49		
	F-06	9.81		
	F-07	8.37		
Negative extraction control		24.38	Ct > 20	Acceptable
Positive control (pSh26-S-Nov)		9.81	Ct ≤ 20	
Negative control (water)		24.44	Ct > 20	

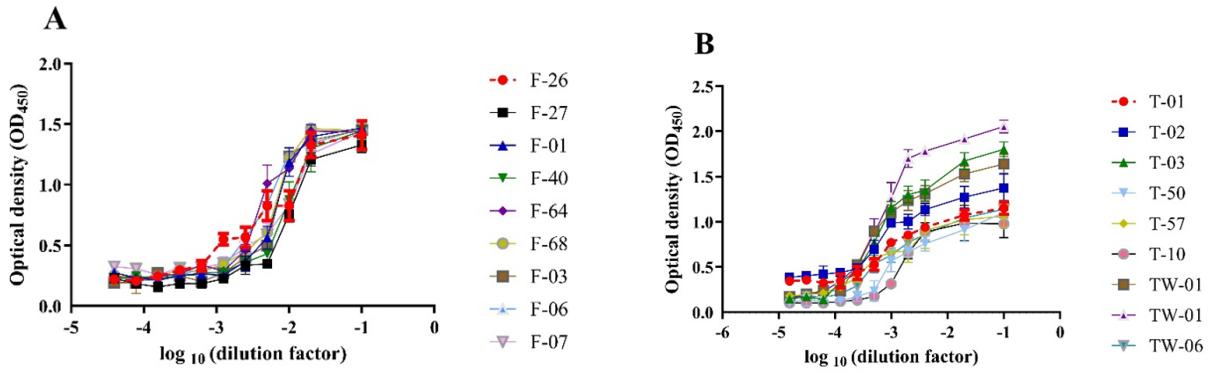

Figure 2. Titration curve. Results of authenticity analysis using an enzyme-linked immunosorbent assay. Optical density measured at wavelength 450 nm. (A) Component I from three manufacturing sites. (B) Component II from three manufacturing sites.

Table 6. Analysis of vaccine samples obtained from three manufacturing sites by the 50% cell culture infectious dose method

Batch	$LogEC_{50}$/мл			Mean $logEC_{50}$/mL	Mean virus titer/mL	Mean virus titer/dose (vp/mL)	Coefficient of variation (%)
Component I							
Manufacturing site 1							
T-01	7.170	7.460	7.362	7.330	2.14×10^7	1.07×10^7	2.013
T-02	7.713	8.207	7.507	7.809	6.44×10^7	3.22×10^7	4.607
T-03	7.509	8.070	7.055	7.544	3.50×10^7	1.75×10^7	6.740
Mean for three batches						2.01×10^7	4.453
Manufacturing site 2							
T-50	7.060	7.055	7.029	7.048	1.12×10^7	0.55×10^7	0.236
T-57	7.269	7.057	7.247	7.191	1.55×10^7	0.77×10^7	1.621
T-10	7.196	7.323	7.861	7.460	2.88×10^7	1.44×10^7	4.732
Mean for three batches						0.92×10^7	2.196
Manufacturing site 3							
TW-01	7.552	7.070	7.529	7.383	2.42×10^7	1.21×10^7	3,683
TW-02	7.716	7.349	7.513	7.526	3.36×10^7	1.68×10^7	2,443
TW-06	7.849	7.134	7.149	7.377	2.38×10^7	1.19×10^7	5,538
Mean for three batches						1.36×10^7	3.888
Mean across manufacturing sites						-	2.23
Component II							
Manufacturing site 1							
F-26	7.688	8.418	8.688	8.264	1.84×10^8	9.20×10^7	6.260
F-27	7.373	7.379	7.801	7.517	3.29×10^7	1.65×10^7	3.264
F-01	7.212	7.420	7.637	7.423	2.65×10^7	1.32×10^7	2.863
Mean for three batches						4.06×10^7	4.129
Manufacturing site 2							
F-40	7.039	7.982	7.687	7.569	3.71×10^7	1.85×10^7	6.373
F-64	7.621	7.645	7.160	7.475	2.99×10^7	1.49×10^7	3.657
F-68	7.834	7.617	7.338	7.596	3.95×10^7	1.97×10^7	3.273
Mean for three batches						1.77×10^7	4.434
Manufacturing site 3							
F-03	8.084	7.631	7.659	7.791	6.18×10^7	3.09×10^7	3.258
F-06	7.888	7.491	7.759	7.712	5.16×10^7	2.58×10^7	2.626
F-07	7.879	7.530	7.600	7.669	4.67×10^7	2.34×10^7	2.408
Mean for three batches						2.67×10^7	2.764
Mean across manufacturing sites							1.38

Table 7. Comparison of virus titer per dose by the 50% cell culture infectious dose method

Source of variation	SS	df	MS	*F*	*p*- value	Conclusion
Component 1						
Between sites	0.167	2	0.0836	2.29	0.182	No significant differences found
Within site	0.219	6	0.0365			
Total	0.386	8			-	
Component 2						
Between sites	0.0678	2	0.0339	0.46	0.652	No significant differences found
Within site	0.441	6	0.0736			
Total	0.509	8			-	

Abbreviations: df: Degrees of freedom; MS: Mean square (sum of squares divided by degrees of freedom); SS: Sum of squares.

methodology.

The acceptance criterion for infectious titer was ≥0.50 × 10^7 vp/mL, in accordance with the product specification. The mean values for all batches complied with this requirement. Standard deviations within each batch set remained low (coefficients of variation < 4.5%), confirming good repeatability of the assay.[25,26,31,33]

The results of the one-way analysis of variance indicate no statistically significant differences among batches from different sites. For the first component (p = 0.182) and the second component (p = 0.652), the probability of differences did not exceed the significance threshold (0.05). These data confirm the comparability of the quality analysis results between the manufacturing sites, demonstrating the reliability of the production technology and the stability of the finished product characteristics.[25,26,31,33]

3.6. Authenticity

Samples from all manufacturing sites were analyzed for authenticity using ELISA. The analysis result is presented in Figure 2.

Based on the results of titrating vaccine Component I and II batches from the three manufacturing sites, an S-shaped dose-dependent sigmoidal curve was observed for all tested samples. This indicates the authenticity of the protein produced by cells following infection with the vaccine preparation across all manufacturing sites.

Table 8 shows the main analytical findings, indicating that product quality remained consistent across all manufacturing sites.[22,31] Each of the assessed attributes, from identity and biosafety to particle concentration and infectious activity, met the established specifications for every batch. Statistical analysis did not reveal meaningful differences between sites, which supports the stability of the process and its ability to deliver reproducible results.

4. Discussion

A comprehensive quality analysis of several batches of the Salnavak vaccine, Components 1 and 2, from three manufacturing sites was conducted. Data were obtained on the quantitative determination of viral particles, authenticity, and safety. The methods are validated and are used in the routine production control of intermediates, active pharmaceutical substances, and the finished drug product of the Salnavak vaccine.

The analysis demonstrated the authenticity of the genetic insert and the expressed protein. This is confirmed by real-time PCR and ELISA. The ability of the obtained vectors to infect cells was analyzed using the $CCID_{50}$ method; the resulting infectious titer ranged from 0.92 × 10^7 to 4.06 × 10^7 vp/mL. The biosafety of the vectors in terms of replication-competent viruses was demonstrated in the PFU assay, where no plaques were detected.[34,35]

A comparison between the two components showed expected, serotype-related differences. Component II, which is based on the Ad5 vector, generally demonstrated a slightly higher infectious titer than Component I (Ad26), a trend that aligns with published observations on the distinct replication characteristics of these serotypes.[34,35]

Across all three manufacturing sites, the variability of key quality attributes remained low. PCR cycle threshold values, viral particle concentrations, and infectivity measurements were highly consistent, and replication-competent adenoviruses were not detected in any batch. Taken together, these results indicate that the production process is well controlled and maintains its performance regardless of site-specific conditions, supporting the

Table 8. Summary of the main quality attributes evaluated for Components I and II across the three manufacturing sites

Quality attribute	Analytical method	Specification/acceptance criterion	Component I (Ad26)	Component II (Ad5)	Overall Conclusion
Identity (genetic insert)	Real-Time PCR (hexon + S-gene detection)	Ct (FAM) < 30; Ct (HEX) ≤ 20; Ct (SYBR) ≤ 20	All batches met criteria; stable Ct values across sites	All batches met criteria; stable Ct values across sites	Authenticity confirmed for all samples
Identity (protein expression)	Biological method with ELISA detection	Sigmoidal dose–response curve	Sigmoidal curve confirmed for all batches	Sigmoidal curve confirmed for all batches	Correct expression verified
Biosafety (RCA absence)	PFU assay on A549 cells	No detectable plaques	No plaques observed in any batch	No plaques observed in any batch	RCA not detected
Total viral particles	Ultraviolet spectrophotometry (A260/A280)	$(1.0 \pm 0.5) \times 10^{11}$ particles/dose	$0.9–1.1 \times 10^{11}$ particles/dose	$0.9–1.2 \times 10^{11}$ particles/dose	Particle content consistent
Infectious titer	$CCID_{50}$ assay	$\geq 0.5 \times 10^{7}$ vp/mL	$0.92 \times 10^{7}–2.01 \times 10^{7}$ vp/mL; CV < 4.5%	$1.77 \times 10^{7}–4.06 \times 10^{7}$ vp/mL; CV < 4.5%	All batches met infectious titer criteria
Inter-site variability	ANOVA	$p > 0.05$ indicates no significant differences	$p = 0.182$	$p = 0.652$	No statistically significant inter-site differences
Overall assessment	—	Compliance with the specification	Fully compliant	Fully compliant	Production process stable and comparable across sites

Abbreviations: ANOVA: Analysis of variance; $CCID_{50}$: 50% cell culture infectious dose; CV: Coefficient of variation; Ct: Cycle threshold; ELISA: Enzyme-linked immunosorbent assay; FAM: Fluorescein amidite; HEX: Hexachlorofluorescein; PFU: Plaque-forming unit; RCA: Replication-competent adenovirus; Real-time PCR: Real-time polymerase chain reaction.

overall stability and robustness of the manufacturing technology.[25,27,28]

The described set of methods demonstrated the authenticity of the particles, their contents, and their ability to infect cells and induce expression of the target protein.[34,35] The convergence of analytical control results between manufacturing sites from batch to batch was also shown in other studies.[35-37]

5. Conclusion

The biological safety of the vaccine produced at different manufacturing sites was demonstrated using the PFU method, in which monolayer destruction was not detected. Additionally, an S-shaped dose-dependent sigmoidal curve generated through ELISA confirms the authenticity of the expressed protein. The infectious titer of the preparation ranged from 0.92×10^7 to 4.06×10^7 vp/mL. The authenticity of the expression cassette was confirmed by real-time PCR, which detected the presence of the SARS-CoV-2 S protein gene. Lastly, the comparison of the obtained data confirms the comparability of the critical quality attributes of the vaccine produced at different manufacturing sites.[29,38,39]

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Conflict of interest

The authors are employees of JSC Generium, the manufacturer of the vaccine evaluated in this study. This affiliation is disclosed as a potential conflict of interest. The authors declare that their employment did not influence the study design, data collection, analysis, interpretation of results, or the decision to publish the findings.

Author contributions

Conceptualization: Daria Yu. Kolomiytseva, Natalia A. Litvinova

Data curation: Daria Yu. Kolomiytseva

Formal analysis: Daria Yu. Kolomiytseva

Investigation: Daria Yu. Kolomiytseva, Anastasia A. Sirotkina

Methodology: Daria Yu. Kolomiytseva

Writing – original draft: Daria Yu. Kolomiytseva

Writing – review & editing: Natalia A. Litvinova

Ethics approval and consent to participate

Not applicable.

Consent for publication

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Availability of data

Data will be made available upon request to the corresponding author.

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