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DEVELOPMENT AND PRELIMINARY ANALYTICAL EVALUATION OF REAL-TIME PCR ASSAYS FOR AFRICAN SWINE FEVER VIRUS DETECTION

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ABSTRACT

African swine fever virus (ASFV) is a large, double-stranded DNA virus that poses a major threat to the pig industry, highlighting the need for reliable molecular diagnostics. This study reports on the design and analytical development of two primer–probe systems targeting conserved ASFV genomic regions (B646L/p72), supported by *in silico* assessment and laboratory testing with synthetic DNA constructs. Classical PCR confirmed amplification of both targets from cloned control templates, producing amplicons of the expected size. In probe-based real-time PCR, consistent amplification was obtained for the ASFV-3 system using a pET-asf-3 clone across serial dilutions (Ct ~27–34), while the ASFV-1 probe-based assay did not generate a detectable signal under the applied conditions. Under the tested conditions, the preliminary limit of detection observed for the ASFV-3 probe-based assay was 10^3 copies per reaction. These results demonstrate the feasibility of the developed approach and indicate that the ASFV-3 system supports further analytical evaluation, while the ASFV-1 system requires additional optimization of probe parameters and/or thermal cycling conditions.

Key words: African swine fever virus, real-time PCR, molecular diagnostics, primer–probe design, preliminary analytical evaluation, synthetic DNA controls.

INTRODUCTION

African swine fever (ASF) is a highly contagious viral disease of domestic pigs and wild suids, characterized by severe clinical manifestations and mortality rates that can approach 100% in acute forms [1]. The disease causes substantial economic losses to the pig industry worldwide and poses a persistent threat to animal health systems due to the absence of licensed vaccines and specific antiviral treatments. Under these conditions, rapid and reliable laboratory diagnosis remains the cornerstone of ASF control and surveillance programs [2, 3].

The causative agent, African swine fever virus (ASFV), is a large, double-stranded DNA virus belonging to the genus *Asfivirus* within the family *Asfarviridae*. The viral genome, ranging from approximately 170 to 193 kbp, exhibits considerable genetic variability, which complicates the development of universally applicable diagnostic tools [4, 5]. The circulation of multiple ASFV genotypes across different geographic regions further increases the risk of false-negative results when diagnostic assays are based on insufficiently conserved genomic targets [6–8].

Molecular diagnostic methods based on polymerase chain reaction (PCR) are widely recognized as reference techniques for ASFV detection due to their high analytical sensitivity and specificity, as well as their ability to detect viral DNA at early stages of infection and in subclinical cases [9]. Real-time PCR assays, in particular, offer advantages in terms of rapid turnaround time, reduced contamination risk, and quantitative assessment of viral load. However, the performance of PCR-based diagnostics critically depends on the selection of appropriate primer–probe systems targeting conserved regions of the ASFV genome [10–12].

Genes encoding the major capsid protein p72 (B646L) and the p54 structural protein are among the most commonly used molecular targets for ASFV detection, as they combine relative sequence conservation with diagnostic relevance. Nevertheless, continuous evaluation and optimization of primer–

probe designs remain necessary to ensure reliable detection across genetically diverse ASFV strains and to maintain assay robustness under routine laboratory conditions [13–16]. Although multi-target and DIVA-compatible assays have become increasingly important in endemic regions and in countries implementing vaccination strategies, Kazakhstan remains free from African swine fever and does not use ASF vaccines. Therefore, the primary objective of the present assay was reliable detection and confirmation of ASFV presence rather than differentiation of vaccine and field strains.

In some regions, including areas currently free from confirmed ASF outbreaks, the lack of available viral isolates presents an additional challenge for diagnostic assay development and validation. In such cases, the use of synthetic DNA constructs representing target ASFV genomic regions provides a practical and biosafe alternative for assay optimization, preliminary analytical evaluation, and the establishment of calibration standards [17].

The aim of the present study was to develop and perform a preliminary analytical evaluation of real-time PCR assays for the detection of African swine fever virus based on conserved genomic regions, using synthetic DNA controls. The work focused on primer–probe design, *in silico* evaluation, optimization of reaction conditions, and preliminary assessment of analytical performance and target amplification, providing a methodological basis for the development of applied diagnostic tools for ASFV detection.

MATERIALS AND METHODS

In silico analysis and target selection

Publicly available African swine fever virus genome sequences were retrieved from the GenBank database (NCBI) and used for comparative sequence analysis. Multiple sequence alignments were performed using the ClustalW algorithm implemented in BioEdit software (version 7.0) to identify conserved genomic regions suitable for molecular

Table 1 - Oligonucleotide sequences of primers and probes for ASFV detection

System	Designation	Sequence (5'–3')	Type	Fluorophore	Quencher
ASFV-1	asfv-1f	CCACGTAATCCGTGTCCCAA	Forward primer	–	–
ASFV-1	asfv-1r	GATGATCCGGGTGCGATGAT	Reverse primer	–	–
ASFV-1	asfv-1p	TCCTCCCGTGGCTTCAAAGC	Probe	FAM	BHQ1
ASFV-3	asfv-3f	TCTCCGGGGTATTTCGCAGTA	Forward primer	–	–
ASFV-3	asfv-3r	TCGCAACGGATATGACTGGG	Reverse primer	–	–
ASFV-3	asfv-3p	GCGTGTAACGGCGCCCTCT	Probe	FAM	BHQ1

detection. Target selection was based on sequence conservation, diagnostic relevance, and established recommendations for ASFV molecular diagnostics. Genomic regions within the B646L (p72) genes of ASFV were selected as targets for assay development [18–19].

Primer and probe design

Primer–probe sets were designed using Primer3 software according to standard criteria: primer length of 18–25 nucleotides, melting temperature (T_m) of 55–65 °C, GC content of 40–60%, minimal self-complementarity, and absence of stable secondary structures or primer–dimer formation. Amplicon lengths were selected within the range of 80 – 320 bp to ensure efficient amplification and compatibility with probe-based real-time PCR detection.

Hydrolysis probes were labeled at the 5' end with the fluorescent reporter dye FAM and at the 3' end with the BHQ1 quencher. The oligonucleotide sequences of the developed primers and hydrolysis probes used in this study are listed in Table 1.

In silico specificity assessment

The specificity of all designed primers and probes was evaluated using Primer-BLAST against the NCBI nucleotide database to exclude potential homology with non-target viral genomes and host DNA. Thermodynamic parameters, secondary structures, and dimerization potential were further assessed using OligoAnalyzer and SnapGene software.

Preparation of synthetic DNA controls

Due to the absence of available ASFV isolates, synthetic double-stranded DNA fragments corresponding to the selected ASFV target regions were designed. Fragments of 129 bp and 146 bp encompassing the primer–probe binding sites were synthesized and cloned into the pET plasmid vector. Recombinant plasmids were used as positive control templates for assay optimization, preliminary analytical evaluation, and calibration experiments. Plasmid DNA concentrations were determined spectrophotometrically, and copy numbers were calculated based on molecular weight. Copy numbers were calculated using Avogadro's constant and plasmid molecular weight. Ten-fold serial dilutions were prepared in nuclease-free water to generate standard templates for analytical sensitivity testing. The designations 10^{-3} and 10^{-4} used throughout the optimization experiments refer to ten-fold serial dilutions of the synthetic plasmid DNA stock solution used as amplification templates.

Real-time PCR conditions

Real-time PCR assays were performed in a total reaction volume of 25 μ L using a DNA-based real-time PCR format (no reverse transcription). Each reaction mixture contained 5 $\times$ PCR buffer, $MgCl_2$, deoxynucleotide triphosphates (dNTPs), forward and reverse primers, a FAM/BHQ1-labeled hydrolysis probe, Taq DNA polymerase, and nuclease-free water.

Amplification was carried out on a real-time PCR instrument under the following conditions: initial enzyme activation at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 10 s and combined annealing/extension at 55 °C for 35 s. Fluorescence signals were recorded in the FAM channel during each amplification cycle. Reaction component concentrations were optimized experimentally.

Optimization of reaction components

Optimization experiments were performed to determine the optimal concentrations of $MgCl_2$, primers. Each reaction component was evaluated independently using ten-fold serial dilutions of synthetic ASFV DNA templates. All reactions were conducted in triplicate, and threshold cycle (C_t) values were used as the primary criterion for performance assessment. During optimization of each component, previously optimized parameters were kept constant.

Preliminary analytical sensitivity and target amplification assessment

Preliminary detection performance was assessed using ten-fold serial dilutions of synthetic ASFV DNA control templates. The preliminary limit of detection was defined as the lowest template concentration consistently detected in all replicate reactions. Based on probe-based real-time PCR results, a preliminary detection limit could be reported only for the ASFV-3 system, as consistent amplification was not obtained for the ASFV-1 probe-based assay under the applied conditions.

Target amplification and no-template control performance were evaluated using classical PCR and probe-based real-time PCR with ASFV synthetic DNA templates and no-template controls. Classical PCR yielded amplification products of the expected size for both ASFV targets. In probe-based real-time PCR, amplification of the target synthetic DNA was observed only for the ASFV-3 system, while no detectable signal was observed in no-template control reactions. Under the tested conditions, these findings demonstrated amplification of the target synthetic ASFV DNA and the absence of detectable signal in the NTC; they were not considered sufficient to establish analytical specificity against non-target biological matrices.

Statistical analysis

Ct values obtained from replicate reactions were expressed as mean values with corresponding standard deviations. Coefficients of variation were calculated to describe variability among replicate reactions. Statistical analyses were performed using standard descriptive statistical methods.

RESULTS

Primer–probe design and *in silico* evaluation

Comparative analysis of ASFV genomic sequences enabled the identification of conserved regions within the B646L (p72) genes suitable for molecular detection. Based on these regions, two primer–probe systems (ASFV-1 and ASFV-3) were designed.

In silico evaluation demonstrated that all designed oligonucleotides met predefined quality criteria, including appropriate melting temperatures, balanced GC content, and the absence of critical secondary structures or primer–dimer formation. The results of *in silico* analysis are summarized in Table 2 and indicate the theoretical suitability of the designed primers and probes for real-time PCR assay development.

Although all primers and probes met the predefined *in silico* design criteria, computational predictions do not guarantee optimal experimental performance. Therefore, subsequent laboratory testing was performed to assess amplification performance and probe functionality under real-time PCR conditions.

Synthetic DNA controls

DNA fragments of 129 bp (ASFV-1) and 146 bp (ASFV-3), encompassing the corresponding primer–probe binding

regions, were successfully synthesized and cloned into the pET vector. These constructs were used as standardized positive control templates for assay optimization and analytical evaluation. The use of synthetic controls enabled controlled evaluation of assay performance in the absence of available ASFV isolates and provided reproducible calibration material for sensitivity testing.

Optimization of real-time PCR reaction conditions

To achieve stable and consistent amplification, key reaction components were optimized experimentally. Optimization of MgCl₂ concentration demonstrated its critical influence on amplification performance and Ct value stability. As shown in Table 3, optimal performance was observed at MgCl₂ volumes of 3.0–5.5 µL per reaction, with minimal coefficients of variation across replicates. No amplification signal was detected at the MgCl₂ concentration of 1.0 µL; therefore, Ct values and associated statistical parameters could not be calculated and are indicated as not determined in Table 3.

Similarly, primer concentration optimization revealed that increased primer amounts resulted in lower Ct values and improved signal stability, with optimal performance observed at 2.0 µL of each primer (10 pmol/µL), as summarized in Table 4. Thus, optimization experiments were performed for all major reaction components, including MgCl₂ concentration (Table 3), primer concentration (Table 4), and DNA polymerase concentration (Table 5). The selected reaction conditions were based on the lowest Ct values combined with acceptable reproducibility across replicate reactions.

Enzyme optimization experiments confirmed that the se-

Oligonucleotide	Secondary structures	Dimerization	Thermal compatibility	Result
asfv-1f	No critical structures detected	Not detected	Compatible	Suitable
asfv-1r	No critical structures detected	Not detected	Compatible	Suitable
asfv-1p	Stable	Absent	Compatible	Suitable
asfv-3f	Not detected	Absent	Compatible	Suitable
asfv-3r	Not detected	Absent	Compatible	Suitable
asfv-3p	Stable	Absent	Compatible	Suitable

Table 3 - Threshold cycle (Ct) values obtained during optimization of MgCl₂ concentration

MgCl ₂ volume, µL (25 mM)	Dilution	Mean Ct value	Standard deviation (σ)	95% confidence interval	Coefficient of variation
1.0	10 ⁻³	—	—	—	—
	10 ⁻⁴	—	—	—	—
2.0	10 ⁻³	27.80	0.64	26.21–29.38	2.30
	10 ⁻⁴	32.74	0.57	31.32–34.16	1.74
3.0	10 ⁻³	23.37	0.69	21.64–25.09	2.97
	10 ⁻⁴	27.49	0.42	26.45–28.53	1.52
4.0	10 ⁻³	22.49	0.25	21.88–23.10	1.09
	10 ⁻⁴	27.80	0.67	26.14–29.45	2.40
5.0	10 ⁻³	23.98	0.16	23.58–24.38	0.67
	10 ⁻⁴	28.57	0.96	26.19–30.95	3.35
5.5	10 ⁻³	23.15	0.24	22.55–23.74	1.03
	10 ⁻⁴	27.24	0.15	26.87–27.60	0.54

Table 4 - Threshold cycle (Ct) values obtained during optimization of primer concentration in real-time PCR.

Primer volume, μL (10 pmol/ μL)	Dilution	Mean Ct value	Standard deviation (σ)	95% confidence interval	Coefficient of variation
0.5	10^{-3}	23.29	0.06	23.15–23.43	0.24
	10^{-4}	30.06	1.06	27.42–32.70	3.54
1.0	10^{-3}	22.50	0.37	21.59–23.41	1.63
	10^{-4}	28.00	0.50	26.75–29.24	1.79
1.5	10^{-3}	22.17	0.21	21.64–22.70	0.96
	10^{-4}	26.87	0.26	26.23–27.51	0.96
2.0	10^{-3}	21.07	0.06	20.93–21.20	0.26
	10^{-4}	24.86	0.20	24.37–25.36	0.80

Table 5 - Threshold cycle (Ct) values obtained during optimization of DNA polymerase concentration in real-time PCR.

Polymerase volume, μL	Dilution	Mean Ct value	Standard deviation (σ)	95% confidence interval	Coefficient of variation
0.25	10^{-3}	22.65	0.66	21.00–24.30	2.93
	10^{-4}	25.79	0.35	24.91–26.67	1.38
0.50	10^{-3}	23.07	0.80	21.08–25.06	3.47
	10^{-4}	29.83	0.74	28.00–31.66	2.48
0.75	10^{-3}	23.87	0.47	22.69–25.05	1.99
	10^{-4}	28.90	0.84	26.80–30.99	2.92

lected DNA polymerase concentration ensured efficient and reproducible amplification of ASFV DNA (Table 5). Across the tested polymerase concentrations, mean Ct values ranged from 22.65 to 23.87 for the 10^{-3} dilution and from 25.79 to 29.83 for the 10^{-4} dilution. Coefficients of variation remained below 3.5%, indicating good assay reproducibility under the evaluated conditions.

Preliminary detection performance

Preliminary detection performance was evaluated using ten-fold serial dilutions of cloned synthetic ASFV DNA templates. Classical PCR with asfv-1f/asfv-1r and asfv-3f/asfv-3r primer pairs produced amplicons of the expected size for both targets, demonstrating the functionality of both primer sets under the tested conditions. In probe-based real-time PCR, consistent amplification was obtained for the ASFV-3 primer–probe system using the pET across serial dilutions (Ct approximately 27–34). In contrast, the ASFV-1 probe-based assay did not generate a detectable signal under the applied conditions, despite successful classical PCR amplification.

Under the tested conditions, a preliminary limit of detection of 10^3 copies per reaction was observed for the ASFV-3 probe-based assay. Further optimization of the ASFV-1 probe parameters and/or thermal cycling profile is required prior to further analytical sensitivity assessment.

Target amplification and no-template control performance

Target amplification of the developed assays was assessed using both classical PCR and probe-based real-time PCR approaches. Classical PCR analysis with ASFV-1 and ASFV-3 primer pairs yielded products of the expected size for the corresponding cloned ASFV fragments.

Probe-based real-time PCR amplification was further evaluated using probe-based detection. The ASFV-3 primer–probe system demonstrated amplification of the target syn-

thetic DNA with characteristic Ct values, while no detectable signal was observed in no-template control reactions. Post-amplification agarose gel electrophoresis demonstrated the presence of an amplicon of the expected size for ASFV-3.

For the ASFV-1 system, agarose gel analysis revealed the presence of an additional higher-molecular-weight band and incomplete amplification of the expected fragment, suggesting that further optimization of probe-based assay conditions is required. It should be noted that preliminary experiments performed using SYBR Green chemistry and conventional PCR demonstrated amplification of the ASFV-1 target sequence. Therefore, the absence of a detectable signal in the probe-based real-time PCR format is likely associated with suboptimal probe performance rather than primer failure. Further optimization of probe sequence, probe concentration, and annealing conditions is required. These findings suggest that the ASFV-1 probe requires further optimization, potentially involving adjustment of probe concentration and annealing temperature.

Overall, under the tested conditions, the ASFV-3 real-time PCR assay demonstrated amplification of the target synthetic ASFV DNA, while no detectable signal was observed in the no-template controls. These findings do not establish analytical specificity against non-target biological matrices and highlight the need for additional evaluation of non-target swine pathogens and host genomic DNA, as well as further optimization of the ASFV-1 probe-based system.

Since the present study was performed using synthetic DNA constructs corresponding to the designed target sequences, mismatch and SNP analyses against circulating ASFV variants were not performed. Evaluation of potential primer–probe mismatches in contemporary ASFV genomes should be included in future optimization studies.

DISCUSSION

The present study demonstrates the feasibility of developing real-time PCR assays for African swine fever virus detection using synthetic DNA constructs as surrogate positive controls. The selected B646L (p72) genomic region has been widely used in ASFV molecular diagnostics because of its relative sequence conservation and diagnostic relevance. The successful amplification of both ASFV targets by conventional PCR confirmed the functionality of the designed primer pairs and supported the suitability of the selected genomic regions for assay development.

Although *in silico* evaluation indicated that both primer-probe systems fulfilled the predefined design criteria, experimental testing revealed substantial differences in assay performance. While the ASFV-3 system produced consistent fluorescence signals across serial template dilutions, the ASFV-1 system failed to generate reliable probe-based amplification despite successful amplification in conventional PCR. These findings emphasize that computational assessment alone is insufficient for comprehensive assay evaluation and that laboratory optimization remains a critical step in the development of molecular diagnostic methods.

Under the tested conditions, the preliminary limit of detection observed for the ASFV-3 assay was 10^3 copies per reaction. Although this sensitivity is lower than that reported for some fully optimized international assays, the present work represents an early stage of assay development performed using synthetic DNA controls rather than clinical or reference ASFV materials. Therefore, the obtained value should be regarded as a preliminary analytical characteristic that may be improved through further optimization of probe design, reaction chemistry, and amplification conditions.

Several limitations should be considered when interpreting the results of this study. First, assay development and analytical evaluation were performed using synthetic DNA constructs rather than ASFV isolates or clinical samples. Second, mismatch and SNP analyses against contemporary ASFV genome sequences were not conducted. Third, a preliminary detection limit was assessed only for the ASFV-3 system because the ASFV-1 probe-based assay required further optimization before comprehensive analytical evaluation. Consequently, the reported performance characteristics should be regarded as preliminary and require further assessment using reference materials and field samples. Fourth, slope, R^2 , PCR efficiency, and assay linearity were not calculated at the current stage of development. In addition, cross-reactivity with non-target swine pathogens and host genomic DNA was not experimentally assessed. Determination of analytical specificity, together with slope, R^2 , PCR efficiency, and linearity, should therefore be included in the next stage of full validation.

CONCLUSION

Two primer-probe systems targeting the ASFV B646L (p72) gene were developed and preliminarily evaluated. Under the tested conditions, the ASFV-3 system demonstrated consistent amplification in replicate reactions and a preliminary detection limit of 10^3 copies per reaction, whereas the ASFV-1 system requires further optimization. These findings

provide a methodological basis for continued assay development, further analytical evaluation, and subsequent full validation.

AUTHOR CONTRIBUTIONS

Conceptualization, R.K.N., E.E.B. and M.M.K.; methodology, R.K.N., E.E.B., S.Y.K. and V.M.S.; validation, E.E.B., A.S.K., P.B.A. and Z.S.A.; formal analysis, R.K.N., E.E.B., S.Y.K. and V.M.S.; investigation, E.E.B., A.S.K., P.B.A., Z.S.A., Z.M., Z.Z.T. and A.S.N.; resources, M.M.K. and V.M.S.; data curation, R.K.N., E.E.B. and S.Y.K.; writing—original draft preparation, R.K.N. and E.E.B.; writing—review and editing, S.Y.K., V.M.S. and M.M.K.; visualization, E.E.B., P.B.A. and Z.S.A.; supervision, M.M.K. and V.M.S.; project administration, R.K.N. and M.M.K. All authors have read and agreed to the published version of the manuscript.

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REFERENCES

1. Dixon L.K., Sun H., Roberts H. African swine fever // *Antiviral Res.* - 2019. –Vol. 165. –P. 34-41. - 10.1016/j.antiviral.2019.02.018.
2. Sánchez-Vizcaino J.M., Mur L., Martínez-López B. African swine fever: an epidemiological update // *Transbound Emerg Dis.* - 2012. –Vol. 59. –P. 27-35 - 10.1111/j.1865-1682.2011.01293.x.
3. Zakharova O.I., Titov I.A., Gogin A.E., Sevskikh T.A., Korennoy F.I., Kolbasov D.V., Abrahamyan L., Blokhin A.A. African Swine Fever in the Russian Far East (2019-2020): Spatio-Temporal Analysis and Implications for Wild Ungulates // *Front Vet Sci.* - 2021. –Vol. 8. –P. 8:723081 - 10.3389/fvets.2021.723081.
4. Rock D.L. Challenges for African swine fever vaccine development-«... perhaps the end of the beginning.» // *Vet Microbiol.* - 2017. –Vol. 206. –P. 52-58 - 10.1016/j.vetmic.2016.10.003.
5. Cappai S., Loi F., Coccollone A., Cocco M., Falconi C., Dettori G., Feliziani F., Sanna M.L., Oggiano A. & Rolesu S. Evaluation of a Commercial Field Test to Detect African Swine Fever // *Journal of Wildlife Diseases.* - 2017. –Vol. 53 (3). –P. 602–606 - 10.7589/2016-05-112.
6. Wang L., Luo Y., Zhao Y., Gao G. F., Bi Y., & Qiu H.-J. Comparative genomic analysis reveals an ‘open’ pan-genome of African swine fever virus // *Transboundary and Emerging Diseases.* - 2020. –Vol. 67(4). –P. 1553–1562 - 10.1111/tbed.13489
7. Shotin A.R., Mazloum A., Igolkin A.S., Shevchenko I.V., Elsukova A.A., Aronova E.V., Vlasova N.N. Alternative approaches to the diagnosis of African swine fever in the Russian Federation in 2017–2021 // *Problems of Virology.* - 2022.

- Vol. 67. - P. 290-303 - 10.36233/0507-4088-112

8. Gallardo C., Soler A., Wozniakowski G., & Perez C. A multi gene-approach genotyping method identifies 24 genetic clusters within the genotype II–European African swine fever viruses circulating from 2007 to 2022 // *Frontiers in Veterinary Science*. - 2023. - Vol. 10. – P. 112233 - [10.3389/fvets.2023.1112850](https://doi.org/10.3389/fvets.2023.1112850)

9. Wang L., Zhang X., Zhou Y., & Li F. Specific Detection of African Swine Fever Virus Variants: Novel Quadplex Real-Time PCR Assay with Internal Control // *Microorganisms*. - 2025. - Vol. 13 (3). –P. 615- 622 - [10.3390/microorganisms13030615](https://doi.org/10.3390/microorganisms13030615)

10. Mazur-Panasiuk N., Woźniakowski G. The unique genetic variation within the O174L gene of Polish strains of African swine fever virus facilitates tracking virus origin // *Arch Virol*. – 2019. - Vol. 164(6). – P. 1667-1672 - 10.1007/s00705-019-04224-x

11. Quembo C.J., Jori F., Vosloo W., Heath L. Genetic characterization of African swine fever virus isolates from soft ticks at the wildlife/domestic interface in Mozambique and identification of a novel genotype // *Transbound Emerg Dis*. – 2018. - Vol. 65(2). – P. 420-431 - 10.1111/tbed.12700.

12. Fernández-Pinero J., Gallardo C., Elizalde M., Robles A., Gómez C., Bishop R., Heath L., Couacy-Hymann E., Fasina F.O., Pelayo V., Soler A., Arias M. Molecular diagnosis of African Swine Fever by a new real-time PCR using universal probe library // *Transbound Emerg Dis*. – 2013. – Vol. 60(1). – P. 48-58 - 10.1111/j.1865-1682.2012.01317.x.

13. Bastos A.D., Penrith M.L., Crucièrè C., Edrich J.L., Hutchings G., Roger F., Couacy-Hymann E. R., Thomson G. Genotyping field strains of African swine fever virus by partial p72 gene characterization // *Arch Virol*. – 2003. – Vol. 148(4). – P. 693-706 - 10.1007/s00705-002-0946-8.

14. Agüero M., Fernández J., Romero L., Sánchez Mascaraque C., Arias M., Sánchez-Vizcaíno J.M. Highly sensitive PCR assay for routine diagnosis of African swine fever virus in clinical samples // *J Clin Microbiol*. – 2003. – Vol. 41(9). – P. 4431- 4439 – 10.1128/JCM.41.9.4431-4434.2003.

15. Tignon M., Gallardo C., Iscaro C., Hutet E., Van der Stede Y., Kolbasov D., De Mia G. M., Le Potier M.F., & Bishop R. P. Development and inter-laboratory validation study of an improved new real-time PCR assay with internal control for detection and laboratory diagnosis of African swine fever virus // *Journal of Virological Methods*. - 2011. – Vol. 178 (1–2). –P. 161–170 - 10.1016/j.jviromet.2011.09.007

16. Alkhamis M.A., Gallardo C., Jurado C., Soler A., Arias M., Sánchez-Vizcaíno J.M. Phylodynamics and evolutionary epidemiology of African swine fever p72-CVR genes in Eurasia and Africa // *PLoS One*. - 2018. – Vol. 13(2). –P. e0192565 - 10.1371/journal.pone.0192565.

17. Zhao, K., Bai, X., Wang, Y., & Li, D. The Development of a Multiplex Real-Time Quantitative PCR Assay for the Differential Detection of the Wild-Type Strain and Gene-Deleted Strains of African Swine Fever Virus // *Animals*. - 2022. – Vol. 12(14), 1754. <https://doi.org/10.3390/ani12141754>

18. Haines F.J., Hofmann M.A., King D.P., Drew T.W., Crooke H.R. Development and Validation of a Multiplex, Real-Time RT PCR Assay for the Simultaneous Detection of Classical and African Swine Fever Viruses // *PLoS ONE*. – 2013. – Vol. 8(7): e71019 - <https://doi.org/10.1371/journal.pone.0071019>

19. OIE (WOAH). African swine fever (infection with African swine fever virus). Terrestrial Manual. Chapter 3.9.1. . - 2022.

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РАЗРАБОТКА И ПРЕДВАРИТЕЛЬНАЯ АНАЛИТИЧЕСКАЯ ОЦЕНКА МЕТОДОВ ПЦР-АНАЛИЗА В РЕЖИМЕ РЕАЛЬНОГО ВРЕМЕНИ ДЛЯ ВЫЯВЛЕНИЯ ВИРУСА АФРИКАНСКОЙ ЧУМЫ СВИНЕЙ**Ниссанова Р.К., Башенова Э.Э.*, Каймолдина С.Е., Строчков В.М., Карабасова А.С., Акшалова П.Б., Абай Ж.С., Макай Ж., Тлегенова Ж.Ж., Нурпенсова А.С., Касенов М.М.***ТОО «Казахский научно-исследовательский ветеринарный институт», Алматы, проспект Райымбека, 223, Алматы, 050016, Республика Казахстан***Автор-корреспондент: eralievna86@mail.ru***АННОТАЦИЯ**

Вирус африканской чумы свиней (АЧС) - это крупный вирус с двухцепочечной ДНК, который представляет серьезную угрозу для свиноводческой отрасли, что подчеркивает необходимость надежной молекулярной диагностики. В этом исследовании сообщается о проектировании и аналитической разработке двух систем праймер-зонд, нацеленных на консервативные участки генома вируса АЧС (B646L/p72), при поддержке оценки *in silico* и лабораторных испытаний с использованием синтетических ДНК-конструкций. Классическая ПЦР подтвердила амплификацию обеих мишеней из клонированных контрольных матриц, в результате чего были получены ампликоны ожидаемого размера. При проведении ПЦР в режиме реального времени на основе зондов была получена устойчивая амплификация для системы ASFV-3 с использованием клона pET-asf-3 в последовательных разведениях ($C_t \sim 27-34$), в то время как анализ на основе зондов ASFV-1 не выявил детектируемого сигнала в применяемых условиях. В исследованных условиях предварительный предел обнаружения для системы ASFV-3 на основе зонда составил 10^3 копии на реакцию. Эти результаты демонстрируют осуществимость разработанного подхода и указывают на возможность дальнейшей аналитической оценки системы ASFV-3, в то время как система ASFV-1 требует дополнительной оптимизации параметров зонда и/или условий термоциклирования.

Ключевые слова: вирус африканской чумы свиней, ПЦР в реальном времени, молекулярная диагностика, дизайн праймер-зонда, предварительная аналитическая оценка, синтетические ДНК-контроли.

ЭОК: 619:578.578.2**АФРИКАЛЫҚ ШОШҚА ОБАСЫ ВИРУСЫН АНЫҚТАУ ҮШІН НАҚТЫ УАҚЫТТАҒЫ ПТР ТАЛДАУ ӘДІСТЕРІН ӘЗІРЛЕУ ЖӘНЕ АЛДЫН АЛА АНАЛИТИКАЛЫҚ БАҒАЛАУ****Ниссанова Р.К., Башенова Э.Э.*, Каймолдина С.Е., Строчков В.М., Карабасова А.С., Акшалова П.Б., Абай Ж.С., Макай Ж., Тлегенова Ж.Ж., Нурпенсова А.С., Касенов М.М.***«Қазақ ғылыми-зерттеу ветеринариялық институты» ЖШС, Қазақстан Республикасы, Алматы, 050016, Райымбек даңғылы, 223***Автор-корреспондент: eralievna86@mail.ru***АНДАТПА**

Африкалық шошқа обасы вирусы (ASFV) - бұл шошқа өнеркәсібіне үлкен қауіп төндіретін үлкен қос тізбекті ДНК вирусы, яғни молекулалық диагностиканың қажеттілігін көрсетеді. Бұл зерттеу синтетикалық ДНК конструкцияларын пайдалана отырып, *in silico* бағалауы мен зертханалық сынақтардың қолдауымен ASFV вирусының (B646L/p72) геномының консервативті аймақтарына бағытталған екі праймер-зонд жүйесінің дизайны мен аналитикалық дамуы туралы хабарлайды. Классикалық ПТР клондалған бақылау матрицаларынан екі нысананың да күшейтілгенін растады, нәтижесінде күтілетін өлшемдегі ампликондар пайда болды. Зондтар негізінде нақты уақыт режимінде ПТР жүргізу кезінде дәйекті сұйылтуларда ($C_t \sim 27-34$) pET-asf-3 клонын пайдалана отырып, ASFV-3 жүйесі үшін тұрақты күшейту алынды, ал ASFV-1 зондтарына негізделген талдау қолданылатын жағдайларда анықталатын сигналды анықтаған жоқ. Зерттелген жағдайларда зондқа негізделген ASFV-3 жүйесі үшін алдын ала анықтау шегі бір реакцияға 10^3 дананы құрады. Бұл нәтижелер әзірленген тәсілдің орындылығын көрсетеді және ASFV-3 жүйесін одан әрі аналитикалық бағалау мүмкіндігін көрсетеді, ал ASFV-1 жүйесі зонд параметрлерін және/немесе термиялық цикл жағдайларын қосымша оптимизациялауды қажет етеді.

Негізгі сөздер: африкалық шошқа обасы вирусы, нақты уақыттағы ПТР, молекулалық диагностика, праймер-зонд дизайны, алдын ала аналитикалық бағалау, синтетикалық ДНК бақылаулары.