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Optimisation of RT-PCR for the detection of rabies virus RNA

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Abstract. The relevance of accurate and rapid laboratory diagnosis of rabies is determined by the high epizootic and epidemiological significance of this disease, its widespread prevalence among wild and domestic animals, as well as the need for timely and effective anti-epizootic and preventive measures. The introduction of molecular genetic diagnostic methods, which reduce the time required for laboratory confirmation of infection and increase the sensitivity of virus detection in the early stages of the disease, is of additional importance. The aim of this work was to develop and optimise a one-step reverse transcription polymerase chain reaction (RT-PCR) designed for the reliable detection of rabies virus RNA in biological material. The study used molecular biological methods, including the selection and analysis of specific primers for the N gene of the rabies virus, as well as the optimisation of the main parameters of the one-step RT-PCR. As a result of the work, RabF_N and RabR_N primers were selected, targeting a conserved region of the rabies virus N gene, which ensured high amplification specificity. Key reaction conditions were investigated and analysed, including primer annealing temperature, $MgCl_2$ concentration, and primer concentration in the reaction mixture. Optimal RT-PCR parameters were established, ensuring stable and reproducible amplification of the target viral RNA fragment. It was shown that the developed protocol allows for the effective

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detection of rabies virus RNA and can be used for laboratory confirmation of the diagnosis. The results confirm the feasibility of using one-step RT-PCR as a rapid and sensitive method of molecular diagnosis. The practical value of the work lies in the possibility of introducing the developed and optimised RT-PCR method into the routine activities of veterinary diagnostic laboratories and reference centres for the diagnosis of rabies and epizootic monitoring

Keywords: diagnosis; N gene; primer; amplification; specificity; sensitivity

Introduction

Rabies is one of the most dangerous zoonotic infections, characterised by acute encephalitis and mortality in animals and humans when clinical symptoms develop. The causative agent of the disease, the rabies virus (*Rabies lyssavirus*, genus *Lyssavirus*, family *Rhabdoviridae*), is an RNA-containing virus with a negative polarity genome that encodes five structural proteins: nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and RNA-dependent RNA polymerase (L) (Wang & Xing, 2024). According to D. Manalo *et al*. (2024), the N gene is a highly conserved region that is critical for identifying genetic clusters associated with rabies transmission in specific regions and animal species, making it an optimal target for molecular diagnostics. The spread of the virus among domestic and wild animals remains a serious problem for veterinary medicine and public health in many countries, including Kyrgyzstan, Kazakhstan and neighbouring regions of Central Asia. In Kazakhstan, according to A. Kabzhanova *et al*. (2023), rabies remains endemic and continues to pose a significant epizootic and epidemiological threat to both domestic and wild animals. The presence of stable natural foci of infection and active circulation of the virus in wild carnivore populations has been confirmed (Kabzhanova *et al*., 2022).

Laboratory diagnostics play a significant role in the surveillance system, as the timeliness of management and preventive decisions depends on their accuracy and efficiency. Traditional methods of laboratory diagnosis of rabies, including direct fluorescent antibody (DFA) reaction to the virus glycoprotein and bioassay on laboratory animals, require considerable time and research under increased biosafety conditions (BSL-2/3). However, the use of DFA may be limited by antigen quality, staff expertise, and tissue sample condition, which reduces the sensitivity of the method, especially when examining autolysed tissues (Gigante *et al*., 2024). Molecular genetic methods, such as reverse transcription polymerase chain reaction (RT-PCR), are recognised by the World Organisation for Animal Health (WOAH) and the World Health Organisation (WHO) as primary methods for the diagnosis of rabies. International guidelines recommend the use of pan-lyssavirus RT-PCR test systems with published validation data for diagnostic testing (WOAH, 2023). RT-PCR provides high sensitivity and specificity, rapid results, standardised reagents and objective criteria for interpreting results.

The development of pan-lyssavirus test systems based on real-time RT-PCR, such as LN34 pan-lyssavirus real-time RT-PCR assay (LN34), has demonstrated excellent diagnostic sensitivity (99.72-100%) and specificity (99.99-100%) compared to direct fluorescent testing (Gigante *et al*., 2024). The use of a combination of degenerate nucleotides, multiplex primers and probes allows high sensitivity and specificity to be achieved in the detection of all known variants of the rabies virus and other types of lyssaviruses. In the work of C. Gigante *et al*. (2025), molecular methods proved effective in diagnosing rabies in samples with varying degrees of degradation, including roadkill material. Molecular diagnostics also play a key role in epidemiological studies, allowing genetic typing of isolates and phylogenetic analysis to understand the spread of the virus and identify the sources of outbreaks (Condori *et al*., 2020; Bautista, 2025). In this regard, the importance of molecular biological methods that provide high sensitivity and specificity for pathogen detection regardless of the stage of the disease is increasing. The aim of this study was to optimise the conditions for classical one-step RT-PCR for reliable and sensitive identification of rabies virus RNA based on the selection of specific primers for the N region of the virus gene and the determination of optimal temperature and time parameters for the reaction, as well as reagent concentrations.

Materials and Methods

The work was carried out at the Research Institute for Biological Safety Problems LLP, QazBioPharm National Holding (Republic of Kazakhstan) in 2024-2025. Primers selected based on sequence analysis in the GenBank (n.d.) database were used to amplify the conserved region N of the rabies virus gene. A pair of primers, RabF_N (5′-CCGTGTACTACAAAGAGAAC-3′) and RabR_N (5′-AGGACACGACACGACGAC-3′), was used in the work. The estimated size of the amplicon was 155 base pairs (bp). The selection and analysis of primer nucleotide sequences was performed using Vector NTI software, which allowed for evaluating their correspondence to the target gene region, temperature characteristics, and potential formation of secondary structures. As a result of primer selection, an amplicon of 155 nucleotide pairs was obtained, corresponding to the calculated fragment of the N gene of the rabies virus. The selected primers demonstrated stable and reproducible amplification of the target site during one-step RT-PCR and did not lead

to the formation of non-specific amplification products or primer dimers. Reaction conditions were optimised by selecting the annealing temperature, Mg^{2+} ion concentration, and primer concentration in the reaction mixture. The established parameters ensured clear visualisation of the amplification product of the specified size and high specificity of rabies virus RNA detection.

RNA from the CVS-11 reference strain of rabies virus, obtained from the institute's microorganism collection, was used in this study. The concentration and purity of viral RNA were determined spectrophotometrically using a NanoDrop 2000 instrument (Thermo Scientific, USA) by measuring absorbance ratios at 260 and 280 nm. One-step RT-PCR was performed in a total reaction volume of 25 µl containing the following components: 2.5 µl of 10× RT-PCR buffer; 0.5 µl of a 10 mM deoxynucleotide triphosphate (dNTP) mixture; $MgCl_2$ at a final concentration of 1.25 mM; 0.5 µl each of forward (RabF_N) and reverse (RabR_N) primers at a final concentration of 20 pmol per reaction; 1.0 µl of enzyme mixture; 1.0 µl of viral RNA template (approximately 10 ng/µl); and nuclease-free deionised water added to a final volume of 25 µl. The thermal cycling conditions were as follows: reverse transcription at 50°C for 15 min; initial denaturation at 95°C for 2 min; 35 amplification cycles consisting of denaturation at 94°C for 15 s, primer annealing at 50-60°C for 30 s (varied during optimisation), and extension at 68°C for 60 s; followed by a final extension at 68°C for 5 min. The reaction mixtures were then held at 4°C. Optimisation of the one-step RT-PCR protocol was performed by varying the primer annealing temperature, $MgCl_2$ concentration, enzyme concentration, and primer concentration. All reactions were carried out using a SuperScript III One-Step RT-PCR System with Platinum Taq DNA Polymerase (Invitrogen, USA). The enzyme mixture ("Enzim mix") supplied with the kit contained reverse transcriptase and Taq DNA polymerase.

To optimise the primer annealing temperature during one-step RT-PCR, a temperature gradient in the range of 50-60°C was used. The use of a gradient mode made it possible to evaluate the effect of annealing temperature on the efficiency and specificity of amplification of the target fragment of the N gene of the rabies virus. In each temperature mode, the intensity of the amplification products, the presence of non-specific bands, and the formation of primer dimers were analysed. To determine the optimal concentration of the "Enzim mix" enzyme complex in the one-step RT-PCR reaction, a concentration range of 0.25 to 1.0 units was selected, which allowed for evaluating the effect of the enzyme amount on the efficiency and reproducibility of the amplification of the target fragment of the N gene of the rabies virus. This range was chosen due to the need to determine the minimum enzyme concentration that ensures stable synthesis of the rabies virus N gene cDNA and subsequent PCR amplification, as well as to prevent possible undesirable effects associated with excessive enzymatic activity.

To optimise the concentration of magnesium ions ($MgCl_2$) in the one-step RT-PCR reaction, a range of concentrations corresponding to 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 2.25, 2.5, and 4.0 mM in the reaction mixture. The $MgCl_2$ concentration was selected to determine the optimal conditions for maximum efficiency and specificity of amplification of the target fragment of the N gene of the rabies virus. The reactions were carried out under identical conditions with only the $MgCl_2$ concentration varying. To optimise the primer concentration in the one-step RT-PCR reaction, testing was carried out in the range of 5-40 pM. The reactions were performed at fixed values of the other parameters, including annealing temperature, $MgCl_2$ concentration, and enzyme complex amount, with only the concentration of RabF_N and RabR_N primers varying. The data obtained were used to select the optimal primer concentration for subsequent RT-PCR settings.

To determine the sensitivity of the primer and probe sets, a series of 10-fold dilutions of complementary DNA (cDNA) from 100 ng to 100 ag in the reaction mixture were prepared. RT-PCR was performed under optimised reaction conditions. Cross-reactivity testing of the developed RT-PCR with RNA/DNA of other viruses was performed: Aujeszky's disease virus (*Pseudorabies* virus, *Herpesviridae*), canine distemper virus (*Paramyxoviridae*), ruminant plague virus (*Morbillivirus*, *Paramyxoviridae*). All RT-PCR reactions were performed in three technical replicates. Each series of experiments included a positive control (RNA of the reference strain CVS-11 of known concentration) and a negative control (deionised water without matrix) to exclude contamination.

Results and Discussion

A pair of primers, RabF_N and RabR_N, was used to amplify the conserved region of the N gene of the rabies virus, ensuring high specificity and sensitivity of the reaction. As a result of a one-step RT-PCR, a clear amplification product of the expected size (155 bp) was formed in all positive samples, which corresponds to the calculated size of the target fragment of the N gene (Fig. 1). As shown in Figure 1, lane 1, containing rabies virus RNA, showed a distinct DNA band 155 bp in length, completely matching the size of the positive control amplicon (lane 2). This indicates the correctness of the selected primers and the effectiveness of the optimised reaction conditions. In the negative control, there were no amplification products, which confirms the absence of contamination of the reaction mixture and the high specificity of RT-PCR. The DNA marker (M) used allowed for clearly identifying the size of the obtained amplicons and confirm the correspondence of the experimental data to the theoretically expected result. The absence of non-specific bands and primer dimers indicates the correct selection of primer

concentrations and the optimal amplification temperature regime. Thus, the use of RabF_N and RabR_N primers ensures reproducible and selective amplification of the N fragment of the rabies virus gene and can be used for reliable detection of the pathogen's RNA in laboratory diagnostics.

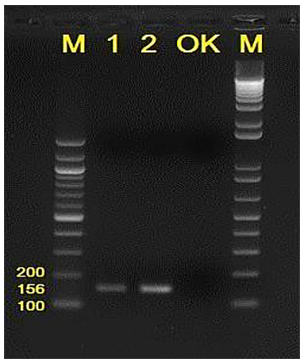

Figure 1. Electrophoregram of the N gene fragment of the rabies virus

Note: M – DNA marker; No. 1 – rabies virus RNA; No. 2 – positive control (155 bp); OK – negative control

Source: developed by the authors

The optimal temperature was found to be 56°C, at which the most intense specific band was formed without by-products and primer dimers. Amplicons become weaker at temperatures above 58°C. The results are presented in Figure 2. The optimal temperature range of 56°C ensured the formation of a clear and reproducible amplicon band of the expected size without the formation of by-products. The selected annealing temperature was used in all RT-PCR reactions in the study. The optimisation of the "Enzim mix" enzyme concentration is shown in Figure 3. RT-PCR results showed that the amplified product was well amplified at concentrations of 0.25-1 units of "Enzim mix" enzyme.

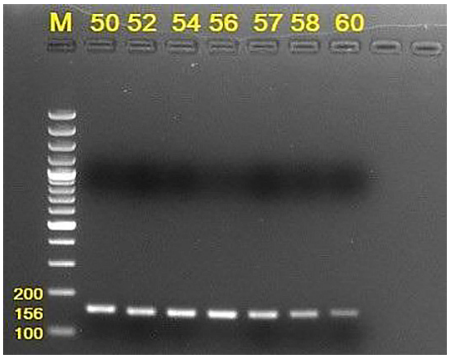

Figure 2. Electrophoregram of amplification of the N fragment of the rabies virus gene at a denaturation temperature gradient (50-60°C)

Note: M – DNA marker

Source: developed by the authors

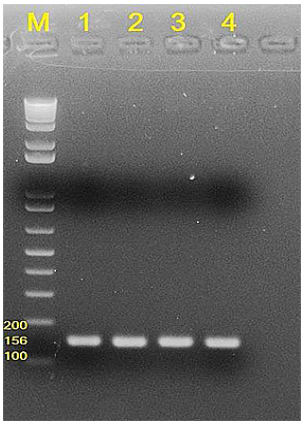

Figure 3. Optimisation of "Enzim mix" enzyme concentration

Source: developed by the authors

The results of RT-PCR optimisation showed that the efficiency of amplification of the target fragment of the N gene of the rabies virus largely depended on the concentration of the "Enzim mix" enzyme complex (reverse transcriptase/DNA polymerase). When testing the range of 0.25-1.0 units of "Enzim mix", a stable and reproducible accumulation of the expected size of the amplicon was observed, which was confirmed by clear visualisation of the band on the electropherogram and the absence of pronounced by-products. A specific amplification product was formed at all specified concentrations, indicating sufficient enzyme complex activity for effective cDNA synthesis and subsequent PCR amplification. When optimising $MgCl_2$, a range of 0.5-4 mM final $MgCl_2$ concentration was tested. The results are shown in Figure 4. The results of optimising the concentration of magnesium ions showed that the use of 1.25 mM $MgCl_2$ in a one-step RT-PCR reaction provides the most stable and reproducible amplification of the target fragment of the N gene of the rabies virus.

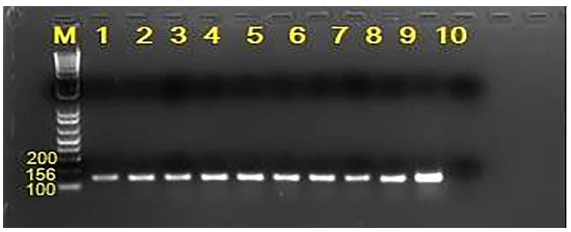

Figure 4. Electrophoregram of product amplification at different $MgCl_2$ concentrations

Note: M – size marker; lanes 1-9 correspond to concentrations of 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.25, 2.5, 4 mM, respectively; at 0.5-4 mM, increasing maximum intensity specific bands are visible

Source: developed by the authors

The optimal result was achieved at 20 pM, at which amplification was stable, intense, and without the

formation of primer dimers (Fig. 5). The use of lower primer concentrations led to a decrease in the intensity of the amplification signal, while an increase in concentration above 20 pM resulted in the accumulation of PCR products. Thus, a concentration of 20 pM was determined to be optimal and was used in all subsequent one-step RT-PCR settings.

Figure 5. Electrophoregram of amplification of the N fragment of the rabies virus gene at different primer concentrations (5, 10, 20, 30, 40 pM)
Note: M – DNA marker; lanes 1-5 correspond to the specified concentrations
Source: developed by the authors

The sensitivity of the method is 10 fg and is comparable to published foreign methods for detecting other lyssaviruses (Faye *et al.*, 2017). This sensitivity is sufficient for the diagnosis of rabies using saliva and brain tissue samples from animals. The results of the sensitivity determination are presented in Figure 6.

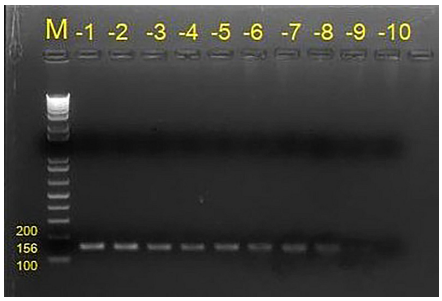

Figure 6. Electrophoregram showing RT-PCR sensitivity at different amounts of rabies virus RNA template
Note: 1 – 100 ng; 2 – 10 ng; 3 – 1 ng; 4 – 100 pg; 5 – 10 pg; 6 – 1 pg; 7 – 100 fg; 8 – 10 fg; 9 – 1 fg; 10 – 100 ag; M – DNA marker
Source: developed by the authors

RT-PCR specificity testing showed that the developed method is specific exclusively for the rabies virus and does not cross-react with the following viruses: Aujeszky's disease virus (*Pseudorabies*, *Herpesviridae*), canine distemper virus (*Paramyxoviridae*), ruminant plague virus (*Morbillivirus*, *Paramyxoviridae*) (Fig. 7).

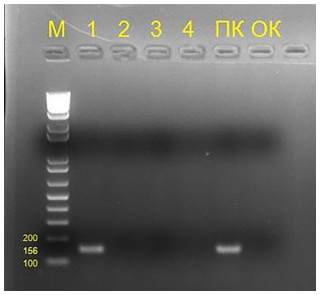

Figure 7. Electrophoregram determining the specificity of RT-PCR
Note: M – DNA marker; 1 – rabies virus RNA; 2 – ruminant plague virus; 3 – Aujeszky's disease virus; 4 – canine distemper virus; ПК – positive control, ОК – negative control
Source: developed by the authors

This study successfully optimised the parameters of a one-step RT-PCR for detecting rabies virus (*Rabies lyssavirus*) RNA using primers targeting the N gene region. The method provides high specificity and sufficient sensitivity for diagnosis. The simplified single-step protocol and the absence of the need for expensive equipment make this test system accessible to veterinary virology laboratories in Central Asian countries. The sensitivity of the method obtained in this study at the level of 10 fg of rabies virus RNA is consistent with data from publications demonstrating the high analytical sensitivity of molecular methods. The study by D. Dean *et al.* (1996) established the effectiveness of DFA, but pointed out its limitations when working with degraded samples, which confirms the relevance of molecular approaches. P. Heaton *et al.* (1997) demonstrated the advantages of semi-nested PCR for the detection of six genotypes of rabies and related viruses, which laid the foundation for the development of modern pan-lyssavirus test systems.

Molecular genetic methods such as RT-PCR are recognised as among the most reliable for the diagnosis of rabies, especially in situations where traditional methods – such as immunodiagnostics or virological testing – may be impossible or ineffective (Ashwini *et al.*, 2024; WOAH, 2024). A study by D. David *et al.* (2002) showed that RT-PCR is capable of detecting the rabies virus in decomposed brain samples that tested negative using a direct fluorescent test, confirming the advantages of molecular diagnostics in complex cases. The development of real-time RT-PCR technologies has significantly expanded the capabilities of laboratory diagnostics. At the same time, it is critical to optimise RT-PCR parameters to ensure the reliability and reproducibility of results. In this study, the optimal annealing temperature was found to be 56°C, the $MgCl_2$ concentration 1.25 mM, the primer concentration 20 pM, and the enzyme complex amount 0.25 units, which is consistent with the recommendations for optimising molecular test systems. D. Manalo *et al.* (2024), when

developing real-time RT-PCR for Philippine strains of rabies virus, also emphasised the importance of optimising the annealing temperature to prevent non-specific amplification, limiting the number of cycles to 35 to improve the accuracy of the analysis.

The specificity of the test system developed in this study was confirmed by the absence of cross-reactivity with other viruses, including Aujeszky's disease virus, carnivore plague virus, and ruminant plague virus. These results are consistent with those of M. Faye *et al*. (2017), who showed no non-specific amplification and cross-reactivity with a wide range of other viruses belonging to the same taxonomic family. The use of conservative regions of the N gene for primer and probe design ensures high specificity while maintaining the ability to detect different variants of the rabies virus. At the same time, as pointed out by Yu. Gavrilova *et al*. (2021), the sensitivity and reliability of molecular systems can vary significantly depending on the quality of the material, the conditions of primer selection, and reaction optimisation.

One important application of molecular diagnostics is expanded epizootic surveillance, including the testing of poor-quality material. C. Gigante *et al*. (2025) showed that real-time RT-PCR successfully detected rabies virus RNA in low-quality roadkill samples, which informed rabies surveillance among wild animals in areas of high interest for rabies control among wild animals. J. Mauhay *et al*. (2023) investigated the possibility of using used lateral flow devices (LFDs) for molecular analysis of rabies virus, demonstrating high sensitivity (96.2-100%) of the LN34 test for rabies diagnosis regardless of the storage temperature of the devices. Molecular epidemiology based on RT-PCR is an important tool for classifying viral diseases in animals, including rabies, and provides a better understanding of epidemiological links (Cliquet *et al.*, 2014). Several aspects are important to consider for the further development of molecular diagnostic methods. M. Ashwini *et al*. (2024) noted in a review of recent updates in laboratory diagnosis of rabies the need to introduce portable PCR platforms and point-of-care tests for field conditions and remote regions with limited resources. P. Wang & L. Xing (2024) emphasised the role of rabies virus structural proteins in evading the immune response and the importance of this understanding for vaccine development, which is also relevant for molecular diagnostics, as mutations in genes can affect the effectiveness of primers and probes.

The data obtained confirm that careful primer selection and optimisation of conditions yield good results even at low viral RNA concentrations, similar to observations in regions with endemic rabies (Caraballo *et al.*, 2021; Faye *et al.*, 2021). The developed RT-PCR test system fits well into the modern practice of molecular diagnosis of rabies, both in terms of sensitivity and specificity, and can serve as a reliable tool for epizootic monitoring and rapid diagnosis. The results of the study demonstrate the high efficiency of the developed RT-PCR test system and confirm the feasibility of widespread use of molecular genetic methods for rapid and reliable detection of pathogens of particularly dangerous zoonotic infections. The use of this approach significantly reduces the time required for laboratory confirmation of the diagnosis compared to traditional methods, which is of fundamental importance for the timely adoption of anti-epizootic and preventive measures. The high sensitivity and specificity of the developed test system make it possible to detect the pathogen even at low concentrations of viral genetic material in the samples under investigation, which is especially important in the early stages of infection and when examining clinical material with limited pathogen content. The method can be recommended for implementation in routine diagnostic practice, as well as for epizootic monitoring of rabies virus circulation in wild animal populations.

Conclusions

During the studies, the optimal conditions for performing a one-step reverse transcription polymerase chain reaction for the detection of rabies virus RNA were experimentally determined. It was found that the best amplification efficiency and specificity were achieved at a primer annealing temperature of 56°C, a magnesium ion concentration of 1.25 mM, an "Enzim mix" enzyme complex concentration of 0.25 units, and a primer concentration of 20 pM. The combination of these parameters ensured stable and reproducible amplification of the target fragment of the N gene of the rabies virus. Under the optimised conditions, a clear amplification product of the expected size was formed without signs of non-specific amplification, which indicates the high selectivity of the developed RT-PCR test system. In negative controls, amplification products were absent, confirming the absence of contamination and the correctness of the reaction setup. The specificity of the test system was confirmed by the absence of cross-amplification when studying other viral samples, indicating that the reaction was directed exclusively at rabies virus RNA.

The sensitivity of the developed method was 10 fg of rabies virus RNA, which indicates the high analytical sensitivity of RT-PCR and the ability to detect viral genetic material at low concentrations in the samples studied. This indicator is of significant importance for early laboratory diagnosis and confirmation of the diagnosis, especially when studying clinical and pathological material with a minimal virus content. The results obtained demonstrate the high efficiency of the developed RT-PCR test system. Thus, the method can be recommended for implementation in the routine diagnostic practice of veterinary laboratories. Prospects for further research include: validation of the developed

protocol on an expanded panel of field isolates of rabies virus from different geographical regions; adaptation of the method in the real-time RT-PCR format using fluorescent probes for quantitative determination of viral load; testing on clinical samples (saliva, cerebrospinal fluid) from animals in the early stages of infection; development of multiplex RT-PCR for the simultaneous detection of rabies virus and other neurotropic viruses.

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Conflict of Interest
None.

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Кутурма вирусунун РНКсын аныктоо үчүн RT-PCRди оптималдаштыруу

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Аннотация. Кутурманы лабораториялык жактан так жана тез диагностикалоонун мааниси бул оорунун жогорку эпизоотиялык жана эпидемиологиялык мааниси, жапайы жана үй жаныбарларынын арасында кеңири таралышы жана эпидемияга каршы жана алдын алуу чараларын өз убагында жана натыйжалуу жүргүзүү зарылдыгы менен аныкталат. Инфекцияны лабораториялык ырастоо үчүн талап кылынган убакытты кыскарткан жана оорунун алгачкы стадияларында вирусту аныктоонун сезгичтигин жогорулаткан молекулярдык-генетикалык диагностикалык ыкмаларды киргизүү кошумча мааниге ээ. Бул изилдөөнүн максаты биологиялык материалдагы кутурма вирусунун РНКсын ишенимдүү аныктоо үчүн ишелип чыккан бир баскычтуу тескери транскриптаза полимераз чынжыр реакциясын (RT-PCR) иштеп чыгуу жана оптималдаштыруу болгон. Изилдөөдө кутурма вирусунун N гени үчүн белгилүү бир праймерлерди тандоо жана талдоо, ошондой эле бир баскычтуу RT-PCRдин негизги параметрлерин оптималдаштыруу сыяктуу молекулярдык биологиялык ыкмалар колдонулган. Бул изилдөөнүн натыйжасында кутурма вирусунун N генинин сакталган аймагына багытталган RabF_N жана RabR_N праймерлери тандалып алынган, бул жогорку амплификациялык өзгөчөлүктү камсыз кылат. Праймерди күйгүзүү температурасы, $MgCl_2$ ионунун концентрациясы жана реакция аралашмасындагы праймердин концентрациясы сыяктуу негизги реакция шарттары изилденип, талданды. Максаттуу вирустук РНК фрагментинин туруктуу жана кайталануучу күчөтүлүшүн камсыз кылган оптималдуу RT-PCR параметрлери белгиленди. Ишелип чыккан протокол кутурма вирусунун РНКсын натыйжалуу аныктай тургандыгы көрсөтүлдү жана диагнозду лабораториялык ырастоо үчүн колдонулушу мүмкүн. Алынган жыйынтыктар бир баскычтуу RT-PCRди тез жана сезгич молекулярдык диагностикалык ыкма катары колдонуунун мүмкүнчүлүгүн тастыктайт. Бул изилдөөнүн практикалык баалуулугу ишелип чыккан жана оптималдаштырылган RT-PCR ыкмасын кутурманы диагностикалоо жана эпизоотиялык мониторинг жүргүзүү үчүн ветеринардык диагностикалык лабораториялардын жана маалымдама борборлорунун күнүмдүк ишине киргизүү мүмкүнчүлүгүндө жатат

Негизги сөздөр: диагностика; N гени; праймер; күчөтүү; спецификалуулук; сезгичтик

Оптимизация ОТ-ПЦР для выявления РНК вируса бешенства

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Аннотация. Актуальность точной и быстрой лабораторной диагностики бешенства определяется высокой эпизоотической и эпидемиологической значимостью данного заболевания, его широкой распространенностью среди диких и домашних животных, а также необходимостью своевременного проведения эффективных противоэпизоотических и профилактических мероприятий. Дополнительную значимость приобретает внедрение молекулярно-генетических методов диагностики, позволяющих сократить сроки лабораторного подтверждения инфекции и повысить чувствительность выявления вируса на ранних стадиях заболевания. Целью настоящей работы являлась разработка и оптимизация одноступенчатой обратной транскриптазной полимеразной цепной реакции (ОТ-ПЦР), предназначенной для надежного выявления РНК вируса бешенства в биологическом материале. В ходе исследования использовали молекулярно-биологические методы, включающие подбор и анализ специфических праймеров к гену N вируса бешенства, а также оптимизацию основных параметров одноступенчатой ОТ-ПЦР. В результате работы были подобраны праймеры RabF_N и RabR_N, направленные на консервативный участок гена N вируса бешенства, что обеспечило высокую специфичность амплификации. Были исследованы и проанализированы ключевые условия проведения реакции, включая температуру отжига праймеров, концентрацию ионов $MgCl_2$ и концентрацию праймеров в реакционной смеси. Установлены оптимальные параметры ОТ-ПЦР, обеспечивающие стабильную и воспроизводимую амплификацию целевого фрагмента вирусной РНК. Показано, что разработанный протокол позволяет эффективно выявлять РНК вируса бешенства и может применяться для лабораторного подтверждения диагноза. Полученные результаты подтверждают целесообразность использования одноступенчатой ОТ-ПЦР как быстрого и чувствительного метода молекулярной диагностики. Практическая ценность работы заключается в возможности внедрения разработанного и оптимизированного метода ОТ-ПЦР в рутинную деятельность ветеринарных диагностических лабораторий и референсных центров для диагностики бешенства и проведения эпизоотического мониторинга

Ключевые слова: диагностика; ген N; праймер; амплификация; специфичность; чувствительность