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Sensitive, Semiquantitative, and Portable Nucleic Acid Detection of Rabies Virus Using a Personal Glucose Meter

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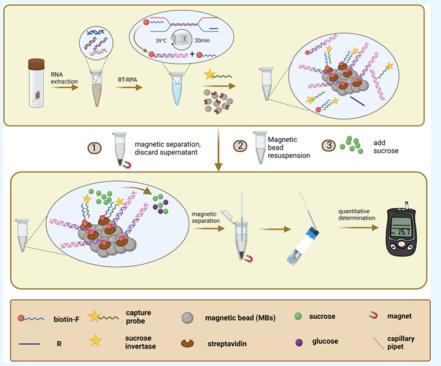
ABSTRACT: Rabies is a zoonotic infection with the potential to infect all mammals and poses a significant threat to mortality. Although enzyme-linked immunosorbent tests and real-time reverse transcription-quantitative polymerase chain reaction (RT-qPCR) have been established for rabies virus (RABV) detection, they require skilled staff. Here, we introduce a personal glucose meter (PGM)-based nucleic acid (NA-PGM) detection method to diagnose RABV. This method ensures sensitive and convenient RABV diagnosis through hybridization of reverse transcription-recombinase aided amplification (RT-RAA) amplicons with probes labeled with sucrose-converting enzymes, reaching a detection level as low as 6.3 copies/μL equivalent to 12.26 copies. NA-PGM allows for the differentiation of RABV from other closely related viruses. In addition, NA-PGM showed excellent performance on 65 clinical samples with a 100% accuracy rate compared with the widely adopted RT-qPCR method. Thus, our developed NA-PGM method stands out as sensitive, semiquantitative, and portable for RABV detection, showcasing promise as a versatile platform for a wide range of pathogens.



INTRODUCTION

Rabies, a serious zoonotic disease caused by rabies virus (RABV), remains a global health concern, contributing to approximately ∼60 000 human deaths annually and imposing a heavy economic burden.[1] The primary mode of transmission is through bites and scratches from various warm-blooded animals carrying the rabies virus, with dogs and cats being the most common.[2,3] Currently, rabies is entirely preventable through vaccination and the most effective prophylaxis measure involves routine vaccination of animal populations susceptible to carrying the virus.[4,5] Despite of success of vaccination, a major challenge arises in free-roaming dogs often being excluded from vaccination campaigns due to the need for administering multiple doses and the associated high cost.[6,7] Thus, rabies has a growing public health impact. Although some animals will be vaccinated against rabies, the limited protective period leaves them vulnerable to subsequent infection. Therefore, timely identification of animals carrying the rabies virus is crucial for prompt vaccination, cutting off the source of transmission and reducing the infection rate.

The traditional virus detection techniques can be categorized into virological, immunological, and molecular methods.[8] The viral culture method is dependent on the large size and well-established database, limiting the development in quick and early detection of viruses.[9] Enzyme-linked immunosorbent assays (ELISA) continue to be the most widely utilized technique for biomarker identification in lab settings.[10] However, the relatively low sensitivity curtails their applications in modern laboratory procedures.[11−13] Furthermore, studies indicate that the human immune system may not be active during the early stages of infection, which could result in false-negative diagnoses.[14] In contrast, the nucleic acid test emerges as a timely and accurate alternative, capable of detecting nucleic acid in the blood within 24 h of infection.[15,16] It is particularly important to note that unlike the viral culture and ELISA methods, nucleic acids tests can be conducted outside traditional laboratory settings.[17,18] Therefore, viral nucleic acid assays are the most practical and valuable for accurate diagnosis of rabies virus (RABV) infection.

With the advancement of variable and isothermal amplification technologies, nucleic acid tests are currently thriving in the field of diagnosis.[19] Some representative variable amplification technologies, like polymerase chain reaction (PCR) and real-time quantitative PCR (qPCR), boast high sensitivity but face limitations as they require.[20,21] Alternatively, an isothermal amplification method exemplified by recombinase aided amplification (RAA) offers a more accessible alternative. RAA can be performed using a simple water bath or heating block to allow testing in outbreak areas

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Table 1. Primers and Probe Sequences of the RABV NA-PGM Assay

	position	sequence (5′−3′)
F	713−742	biotin-ACTGGTATCATTTACAGGGTTCATAAAGC
R	980−1009	CATGGGGAGCACATGCGGCAATAACTGTCG
P		SHC6TTTTTTTTTTTTGCTTTATGAACCCTGTAAATGATAC

where large power supplies are not available.[22] In addition, the integration of an electrochemical biosensor for amplicon detection enhances the applicability of nucleic acid testing.[23] To date, RAA-based viral assays have been developed for the detection of various viruses including HPV, mpox virus, and zika virus, among others.[24−26] However, it is difficult to realize quantitative or semiquantitative visual detection of pathogens through the interpretation of terminal data.

In this study, we designed an RAA-based assay for the portable, semiquantitative, and accurate detection of viral nucleic acids. Leveraging a personal glucose meter (PGM)-based nucleic acid (NA-PGM) detection method, this system involves the hybridization of reverse transcription-recombinase aided amplification (RT-RAA) amplicons with probes labeled using sucrose-converting enzymes. The assay demonstrated a sensitivity capable of detecting as few as 6.3 copies/μL RABV RNA. Furthermore, 65 clinical samples were analyzed using NA-PGM with 100% performance rate, which is consistent with the widely adopted real-time reverse transcription-quantitative polymerase chain reaction (RT-qPCR) method. Thus, the sensitive, semiquantitative, and portable NA-PGM system for RABV detection was developed, providing a promising and general platform for a wide range of pathogens.

EXPERIMENTAL SECTION

Synthesis of Primers and Probes. To date, there are no well-designed primer design programs or automated software programs for RAA primers. Current primer design software for PCR, such as Primer 3, is not suitable for RAA primer development because of the longer sequence lengths of RAA primers.[27,28] To establish a nucleic acid detection based on the PGM (NA-PGM) method for the detection of RABV, we downloaded the strain sequences from GeneBank datebase and used MEGA 11 software to compare the conversed N gene sequences of several RABV strains that were collected from various places in different years (Figure S1).[29] Then, we constructed forward primer (F), reverse primer (R), and a probe (P) suitable for RABV NA-PGM (Table 1) using the conserved region of the N gene as a template by Primer Explorer V51 software. Biotin is labeled at the 5′ end of F and P that is complementary to F with thiol modified at the 5′ end. Hereinafter, the capture probe was formed by coupling sucrose invertase to the 5′ end of the probe through a coupling agent. All primers and probes were synthesized by Sangon Biotech (Shanghai, China).

Recombinant Plasmid and Viral RNA. A pcDNA3.1 (+) recombinant plasmid containing the RABV *N* gene was measured using a spectrophotometer available in our laboratory. The copy number of the recombinant plasmid was calculated using the following formula: copies/μL = $6.02 \times 10^{23} \times 10^{-9} \times$ concentration/(fragment length $\times$ 660). With a recombinant plasmid concentration of approximately 340 ng/μL, this calculation yielded a copy number of 6.13×10^{10} copies/μL. According to this, we prepared 10-fold dilutions of the recombinant plasmid ranging from 10^6 to 10^0 copies/μL.

RABV CVS 11 and SRV 9 were cultured in mouse neuroma cells and cell cultures were collected; viral titers were 5×10^6 $TCID_{50}$/100 μL and 2×10^7 $TCID_{50}$/100 μL (build by predecessors of our team). The viral solution was extracted using a TIANamp RNA Kit for Virus detection (TIANGEN, Beijing, China) and stored at −80 °C in a refrigerator.

DNA/RNA of canine adenosis virus (CAV), canine parvovirus (CPV), feline parvovirus (FPV), canine coronavirus (CCV), and canine parainfluenza virus (CPIV) was extracted using the Magnetic Viral DNA/RNA Kit (TIANGEN, Beijing, China) and stored at −80 °C.

RAA Assay. The reactions were prepared following the manufacturer's instructions for the RT-RAA nucleic acid amplification kit (Jiangsu Qitian Gene Biotechnology Co., Ltd., China). Briefly, 50 μL reaction mixtures contained 25 μL buffer V, 2.4 μL forward and reverse primers (10 μM each primer), 15.7 μL master mix of DEPC, 2.5 μL of 280 mM $Mg(CH_3COO)_2$, and 2 μL template. The premixed solution combined with a freeze-dried powder containing RAA was added into the reaction tube. Following mixing and centrifugation, the tubes were placed in a small metal bath at 39 °C for 20 min for amplification.

Detection of RABV Based on NA-PGM. After thorough mixing, 20 μL of BioMag Plus Streptavidin beads (Bang Laboratories, Indiana, USA) was added into a 1.5 mL centrifuge tube. Subsequently, the tube was placed on a magnetic holder for magnetic separation, and the supernatant was discarded. The beads were washed three times using 200 μL of 0.1 M PBS. After the RAA amplification was finished, the product was transferred to the test tube and sat at room temperature for 30 min. 500 μL of 1% BSA solution was added, and the mixture was incubated for 30 min at room temperature to complete the closure procedure. Following the addition of 1 μL of the detection probe and 40 μL of a 0.1 M PBS solution, the mixture was allowed to sit at room temperature for 2 h. After the completion of each of the above steps, the tubes were placed on a magnetic rack, subjected to magnetic separation, discarding the supernatant, and washed three times with 200 μL of 0.1 M PBS. Following addition of 50 μL of 0.5 M sucrose solution, the reaction was finished and catalyzed in a water bath for 30 min at 37 °C. To determine whether or not RABV RNA was present, the concentration of glucose was finally tested using PGM. For every test, a negative control was established and performed three times.

Optimization of the Reaction Conditions for NA-PGM. To determine the ideal closure time, the responses were characterized at two distinct blocking times (0.5 and 1 h). Next, at the ideal blocking time, two distinct probe incubation times (1 and 2 h) were optimized. After that, three different sucrose invertase catalytic temperatures (37, 42, and 55 °C) as well as three different catalytic durations (10, 20, and 30 min) were examined. For every condition mentioned above, the experiment was conducted three times.

Sensitivity and Specificity of the NA-PGM Assay. The plasmid and RNA transcripts were subjected to a 10-fold serial

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dilution. The dilution concentration range of the plasmids was 6.13×10^6–6.13×10^0 copies/μL, and the dilution concentration range of the plasmids containing CVS 11 and SRV 9 N genes was 5.76×10^6–5.76×10^0 copies/μL and 2.28×10^6–2.28×10^0 copies/μL, respectively. These dilutions were used as templates for assessing the sensitivity of the NA-PGM test. Each experiment was repeated three times.

DNA/RNA of canine adenosis virus (CAV), canine parvovirus (CPV), feline parvovirus (FPV), canine coronavirus (CCV), and canine parainfluenza virus (CPIV) was extracted using the Magnetic Viral DNA/RNA Kit (TIANGEN, Beijing, China) and stored at −80 °C. The nucleic acids were used as a template for amplification under the optimal conditions of the NA-PGM assay. Simultaneously, RABV RNA was measured as a positive control, and each trial included three replicates.

RT-qPCR for Detection of RABV RNA. Following the protocol established by Ashutosh Wadhwa et al.,[30] a TaqMan RT-qPCR method was performed for the detection of N gene. After 10-fold dilution, the final concentration of the standard plasmid ranged from 6.13×10^6 to 6.13×10^0 copies/μL. According to the manufacturer's instructions for TransScript Probe One-Step RT-qPCR SuperMix (TransGen Biotech, Beijing, China), each 20 μL reaction mixture contained 10 μL SuperMix, 5.8 μL DEPC-treated water, 0.4 μL 10 μM F and R, 1 μL 10 μM probe, 0.4 μL enzyme mixture, and 2 μL template. Amplification reactions were performed using a StepOnePlus Real-Time PCR system (Thermo Fisher, USA) with cycles set at 94 °C for 30 s, followed by 40 cycles at 94 °C for 5 s, and 60 °C for 30 s. The required primers and probes are shown in Table 2. All primers and probes were synthesized by Shanghai Sangon Biological Engineering Co., Ltd.

Table 2. Primers and Probe Sequences for RABV RT-qPCR Assays

	position	sequence (5′–3′)
F	1–24	ACGCTTAACAACAAAATCAGAGAAG
R	140–164	CTGGGTACTTGTACTCATATTGATC
P	59–75	(FAM) AACACCCCTACAATGGA (BHQ1)

Clinical Samples Evaluation. Some tissues from mice infected and uninfected with RABV were collected, including 50 brain tissues and 15 muscle tissues. Samples from experimental and control groups were performed using both NA-PGM and RT-qPCR. The experimental group is the nucleic acid sample mentioned above, and the control group is the positive plasmid sample. Three replicates were analyzed in each trial.

RESULTS AND DISCUSSION

Principle of NA-PGM Detection. The specific pathway for nucleic acid detection is shown in Figure 1. The RT-RAA reaction facilitates rapid amplification of a 10-copy pathogen gene fragment at constant temperature (39 ± 3 °C) until the product reaches a detectable concentration. Since we labeled biotin at the 5′ end of the F, both the double-stranded amplified fragments and the F not involved in the reaction were labeled with biotin. The P is complementary to F, and the sucrose invertase is coupled to the 5′ end of the probe by a coupling agent as the capture detection probe. Because streptavidin binds specifically to biotin, the inclusion of streptavidinized magnetic beads allows for the capture of biotin-labeled F and amplicons.[31,32] In this case, the double-stranded amplicon is unable to bind complementarily to the detection probe. Meanwhile, F is not engaged in the amplification reaction and can bind complementarily to the probe and attach to the magnetic beads.

Finally, an appropriate amount of sucrose is added, which can be converted to glucose under the action of sucrose-converting enzyme.[33,34] We can indirectly detect the concentration of target nucleic acids by measuring the concentration of catalytically produced glucose with a glucometer. In the NA-PGM method, the amount of unengaged F is proportional to the amount of captured sucrose convertase. Therefore, the higher the target concentration, the lower the detected glucose signal and the lower the final reading result. It also shows that the detection platform that we have built works in a signal-off manner.

Optimizing the NA-PGM Reaction Conditions. To explore the optimal experimental conditions for the NA-PGM platform, we first optimized the reaction conditions by using a recombinant plasmid as a template. To screen the optimal closure time, the reactions were performed under two different time conditions (0.5 and 1 h). When blocking for 0.5 h, the glucose values produced by plasmids at concentrations of 6.13×10^1–6.13×10^5 copies/μL exhibited a range of 28.9–5.7 mmol/L, while the glucose values for 1 h of closure ranged from 22.4 to 9.5 mmol/L (Figure 2A). The results showed that there was no significant difference among the three replications. However, the blocking time of 1 h demonstrated a smaller variance of 0.5 h, and the range of values was smaller. This observation indicated the superiority of the closure time of 0.5 h as the optimal condition for the NA-PGM test.

To verify the optimal incubation time of the probe, we conducted an assay on the recombinant plasmid under two different incubation time conditions (1 and 2 h) at the optimal closure time described above. The glucose values produced by the plasmids ranged from 20.5–2.3 mmol/L for 6.13×10^1–6.13×10^5 copies/μL concentration at 1 h incubation time and from 28.3 to 6.6 mmol/L at 2 h incubation time, with other conditions remaining unchanged and only the incubation time of the probes changed (Figure 2B). Since the peak value at 1 h was higher and closer to the limit value measured by PGM, the difference in glucose concentration corresponding to different plasmid concentrations was greater. Therefore, we established a probe incubation time of 1 h as the optimal incubation time. This has a good optimization effect on the experimental validation of the subsequent steps and improves the peak conditions, especially for sensitivity exploration.

In the course of our experiments, we observed a significant influence on the final glucose values based on the sucrose catalysis time and temperature. For this reason, we set three different sucrose catalytic times (10, 20, and 30 min), allowing each condition to undergo catalysis in the water bath. The best results can be observed for a catalytic time of 30 min, with other conditions remaining unchanged (Figure 2C). After the optimal catalytic time of 30 min was determined, this condition was left unchanged, and three other tests with different sucrose catalytic temperatures (37, 42, and 55 °C) were examined. As shown in Figure 2D, the stability and linearity of the PGM response was least favorable at 55 °C, while the best value was achieved at 42 °C. The variations in catalytic time and temperature, as well as the difference in glucose values, may be due to several reasons. First, the NA-PGM method requires sucrose-converting enzymes to convert sucrose to glucose, and sucrose-converting enzymes show the

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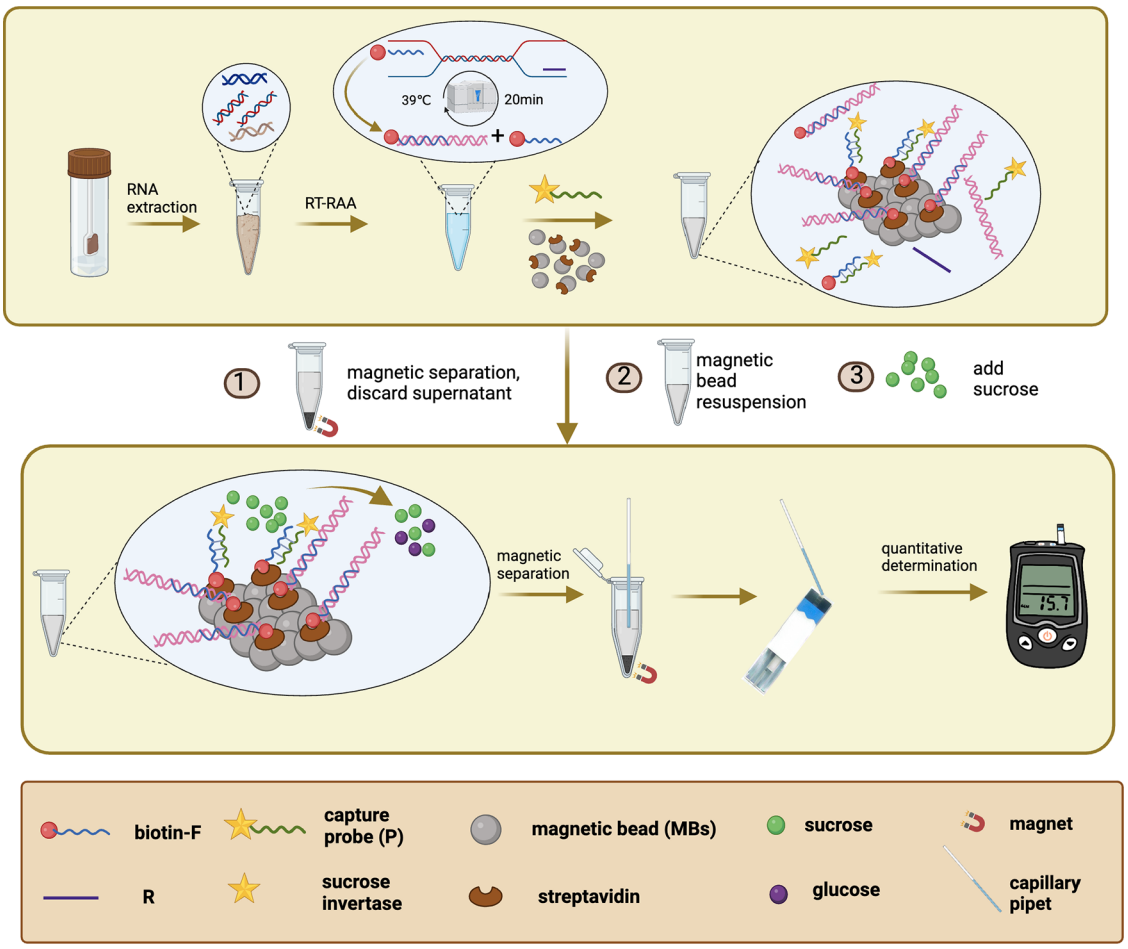

Figure 1. Principles of the NA-PGM method for RABV detection.

highest activity at 42−50 °C.[35] Therefore, keeping the temperature moderate may enhance the effect when the sucrose-converting enzyme acts on sucrose. Second, the optimal catalytic time of sucrose-converting enzyme is 3 h. Although the maximum time set in this experiment is 30 min, which is far from the optimal catalytic time of the sucrose-converting enzyme, the data vary greatly after 10 min. The glucose detection results at 30 min reached the maximum detection limit of glucose meter detection. As a result, the condition is determined as 30 min.

Sensitivity and Semiquantitative Assay of the NA-PGM Platform for RABV Detection. Since there are currently no procedures or strict regulations for RAA-based assays, the most challenging aspect in developing our platform, aimed at maximizing assay sensitivity, was the strategic design of primer pairs capable of amplifying low copy numbers. Based on the comparison, we selected the N gene for the primer pair design. Subsequently, to validate the sensitivity of the NA-PGM platform, we conducted tests with different concentrations of nucleic acids (Figure 3A−C). As the RABV concentration increases from 10^0 to 10^6 copies/μL, we can visually observe a gradual decrease in the value displayed by the PGM. When utilizing the positive plasmid as the RAA template, our method achieved its lowest detection limit at 6.13 copies/μL, equivalent to 12.26 copies; For CVS 11 and SRV 9, the corresponding limits were 5.76 and 9.12 copies/μL, with absolute copy numbers of 11.52 and 18.24 copies. Sheng Ding et al. performed nucleic acid detection of viruses against another common detection method of isothermal amplification, the loop-mediated isothermal amplification (LAMP) method.[36] Our established NA-PGM can detect N RNA down to less than 10 copies, and their results show that the LAMP method can only detect 100 copies of gene N RNA, much lower than our established NA-PGM.

In order to determine the criteria for semiquantitative analysis, we need to establish the corresponding values for the dilution of standard plasmids. Therefore, we performed preliminary experiments involving Taqman RT-qPCR and NA-PGM for different concentrations of recombinant plasmids to determine the corresponding threshold (CT) and PGM values (Pv) prior to formal clinical sample testing. As shown in Table 3, we can classify a negative or positive sample based on the CT and Pv values. A CT value greater than 35 is determined to be negative, while the CT value less than 35 is determined to be positive. Similarly, a Pv value greater than 30 is determined to be negative, while a Pv value less than 30 is determined to be positive. Moreover, the Pv demonstrated a good linear relationship with the detection concentration, which can realize semiquantitative analysis (Figure S2). Following the determination of the infectious status, the values can also provide insight into the extent or range of infection.

In terms of sensitivity, NA-PGM is comparable to RT-PCR, and the combination of RT-RAA and PGM confers a simpler and sensitive signal output for RABV detection. In addition, our platform shows many advantages over other methods, with

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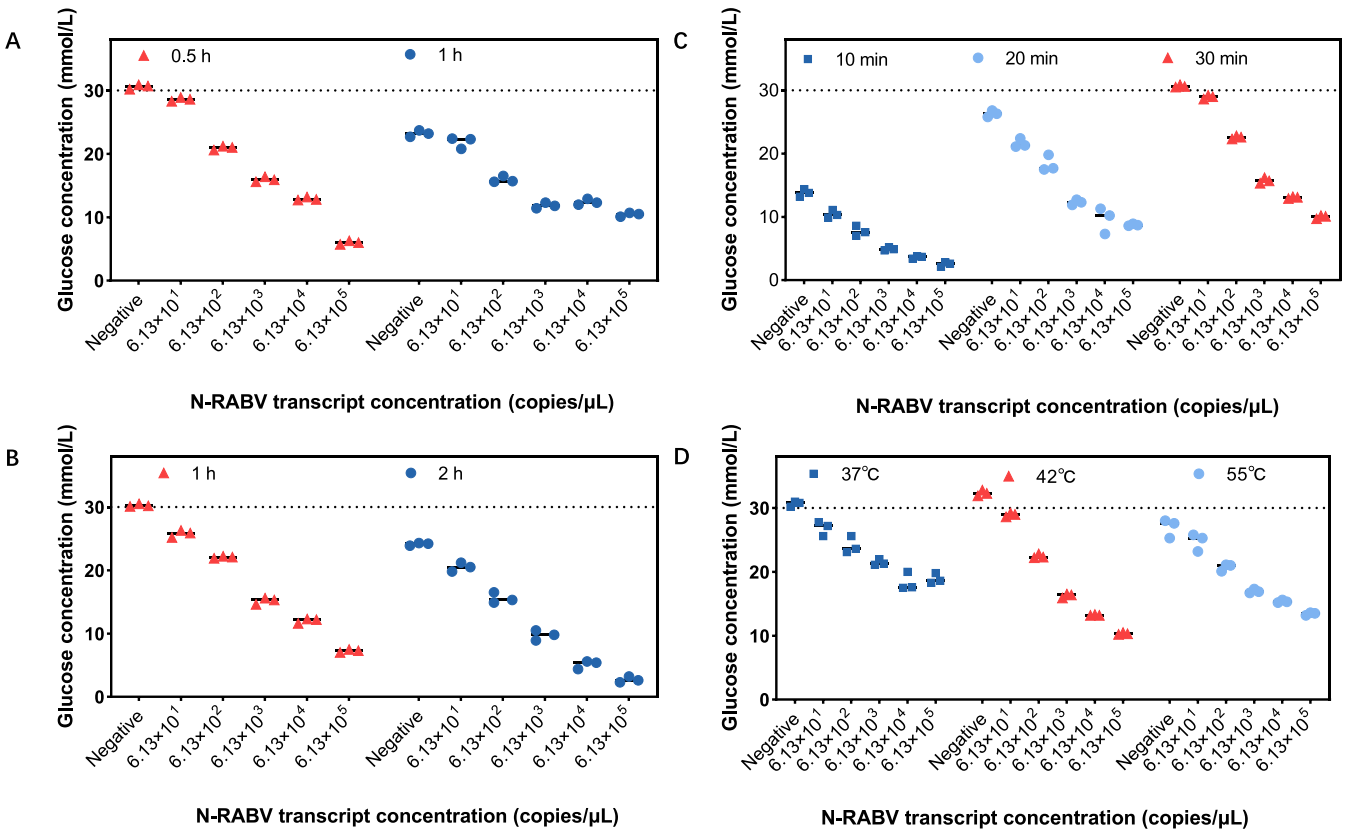

Figure 2. Optimization of NA-PGM method conditions for RABV detection. (a) Optimized conditions for NA-PGM blocking time. (b) Optimized conditions for incubation time of NA-PGM assay probes. (c) Optimized conditions for NA-PGM sucrose catalytic time. (d) Optimized conditions for NA-PGM sucrose catalytic temperature.

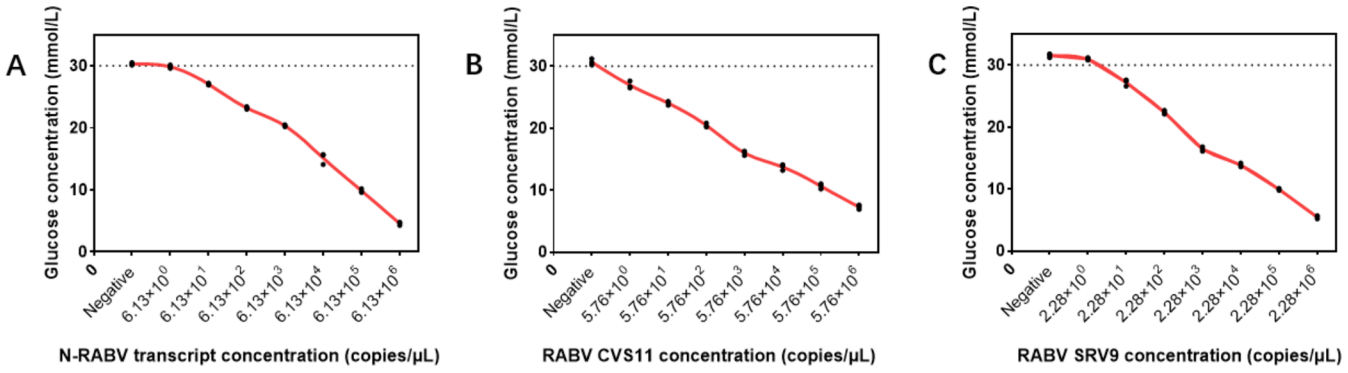

Figure 3. NA-PGM sensitivity testing with different templates. (A) The positive plasmid was used as a template. The recombinant plasmid containing CVS11 N gene (B) and SRV 9 N gene (C) was used as a template.

high sensitivity and a low detection cost. Particularly noteworthy is our method fits well with current testing needs as it can be easily implemented with minimal equipment and training in remote areas where the disease is real and underestimated.[37−39]

Specific Assay of the NA-PGM Platform for RABV Detection. Specificity is a key feature of the detection platform. As RABV clinical symptoms are difficult to identify, a stable testing platform capable of differentiating from other infections is urgently needed to avoid misdiagnosis. In Figure 4, validation of the specificity of the NA-PGM platform was carried out using nucleic acids of multiple viruses, including CAV, CPV, FPV, CCV, and CPIV, all of which are common canine-transmitted viruses. According to previous results, positive samples are expected to exhibit a PGM reading of less than 30 mmol/L, while negative samples should have a reading of more than 30 mmol/L. For this assay, only RABV had a significant positive test result, while the other viruses did not detect the presence of the target. It is worth noting that our amplification primer design was based on RABV strains distributed globally in recent decades. This strategic approach enhances the applicability of our platform in RABV field nucleic acid detection, allowing the detection of multiple RABV strains.

Performance of the RABV NA-PGM Assay on Clinical Samples. The application in clinical sample detection is important for the practicality of our developed method. To enable NA-PGM to be more persuasive in field testing, we

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Table 3. RT-qPCR and NA-PGM Pre-Experiments Results

		CT value				Pv		
different concentrations of cDNA samples	RT-qPCR (CT value)	mean value	standard deviation value	RT-qPCR (CT range)	NA-PGM (Pv)	mean value	standard deviation value	NA-PGM (Pv range)
10^6	15.26, 14.68, 15.23	15.06	0.32	CT < 16	5.6, 6.9, 5.8	6.1	0.70	5 ≤ PV < 9
10^5	18.36, 19.28, 18.65	18.76	0.47	16 ≤ CT < 20	10.2,11.3, 11.5	11.0	0.70	9 ≤ PV < 14
10^4	21.22, 20.89, 21.96	21.35	0.55	20 ≤ CT < 22	15.8, 15.6, 16.2	15.9	0.31	14 ≤ PV < 17
10^3	24.65, 23.22, 23.69	23.85	0.73	22 ≤ CT < 25	20.2, 20.6, 20.5	20.4	0.21	17 ≤ PV < 21
10^2	27.78, 26.97, 27.34	27.36	0.41	25 ≤ CT < 29	24.6, 24.0, 23.9	24.2	0.38	21 ≤ PV < 25
10^1	31.22, 31.27, 30.59	31.03	0.39	29 ≤ CT < 32	27.6, 27.3, 27.5	27.5	0.15	25 ≤ PV < 28
10^0	34.32, 34.26, 33.58	34.05	0.41	CT < 35	28.3, 28.7, 28.1	28.4	0.31	28 ≤ PV < 30
negative	35.02, 35.21, 35.06	35.10	0.11	CT ≥ 35	30.2, 30.3, 30.2	30.2	0.06	PV ≥ 30

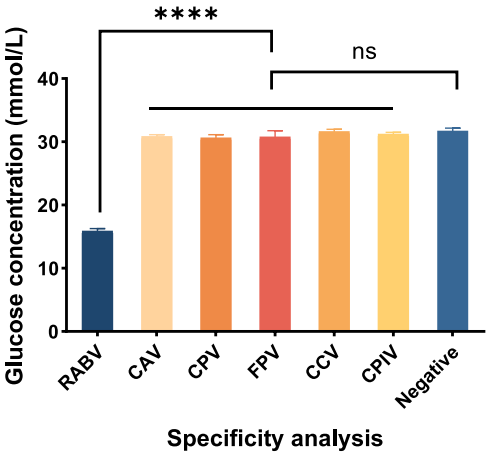

Figure 4. Specific assay of the NA-PGM method.

collected 65 clinical samples, including 50 mouse brain tissue samples and 15 mouse muscle tissue samples, to perform nucleic acid extraction procedures.

The clinical samples were tested by RT-qPCR and then validated by NA-PGM. As shown in Table S1 and Figure 5, the results of both the NA-PGM assay and the RT-qPCR were 60 positive samples and 5 negative samples, which showed a high degree of homogeneity, with a 100% (Kappa = 1) compliance rate between the two methods. These findings indicate that our established NA-PGM method matches the commonly used RT-qPCR method and holds significant clinical applicability, which is an important indicator for continuation of our study.

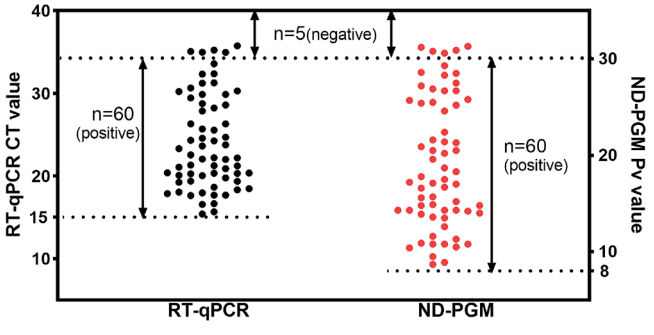

Figure 5. RT-qPCR and NA-PGM results of the clinical samples.

Based on the clinical outcomes, the performance of RT-qPCR and NA-PGM is comparable and suggests the possibility for all veterinarians, veterinary officials, and even individuals in low-resource environments to interpret results without the need for additional equipment manipulation. Although RAA has not so far been recognized as a confirmatory diagnostic method with PCR or qPCR and still requires extensive validation, it presents a potentially convenient and reliable strategy for early diagnosis. Early detection is crucial for subsequent timely response, encompassing treatment, disinfection, early warning measures, and more.

Nowadays, ELISA is still a widely used method in laboratories. However, the insufficiently low catalytic activity and poor stability of natural enzymes together determine that traditional ELISA methods have high detection limits, are highly susceptible to false positives, and are not suitable for home self-testing.[40] RT-qPCR is currently one of the most commonly used methods for detecting RABV, which has high sensitivity and can be applied to most viral assays. But this method requires large and expensive instruments as well as professionally trained staff. Although many miniature qPCR systems have been developed in recent years, they still face problems such as high power consumption and so on. During epidemics, both methods may suffer from their own shortcomings and may not be well suited to field detection. Our NA-PGM detection platform can break through the technical defects of high power consumption and easily produce false positives.

This experimental method combines RAA and PGM. RAA can be operated at room temperature and can even be heated with the palm of the hand or hand warmer pouch[41] with low power consumption. And there have been researchers and scholars who have designed a detection kit for RAA that can be adapted for field testing.[42] PGM is a terminal reading device that is now in people's homes and has successfully begun the road to commercialization. After our calculation, it takes 4 h to test each sample, and the cost of testing each sample is approximately $6. And this experimental method has no limitation of application scenarios, which can more closely fit the detection needs of RABV endemic areas.

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CONCLUSIONS

In summary, we have developed a nucleic acid (NA-PGM) detection method utilizing a personal glucose meter (PGM) for the sensitive, rapid, and convenient diagnosis of RABV. This method involves hybridization of reverse transcription-recombinase aided amplification (RT-RAA) amplicons with probes labeled with sucrose-converting enzymes, achieving detection as low as 6.3 copies/μL. RABV can be accurately distinguished from other closely related viruses. In addition, our NA-PGM demonstrated excellent performance with 65 clinical samples, achieving a 100% accuracy rate, in line with the widely adopted RT-qPCR method. Thus, the sensitive, semiquantitative, and portable NA-PGM system for RABV detection was developed, offering a promising and general platform for the wide array of pathogens.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c01352.

Primers design; NA-PGM semiquantitative assay; and clinical samples evaluation (PDF)

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Notes

The authors declare no competing financial interest.

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