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Research article

Detection of equine herpesvirus type 1 (EHV-1) by recombinase polymerase amplification

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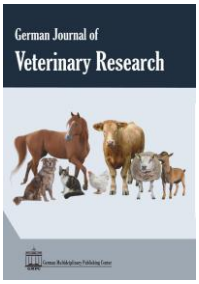

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Abstract

Equine herpesvirus type 1 is a major pathogen of horses, which must be detected quickly and effectively to isolate infected animals and prevent transmission to healthy horses. Laboratory diagnosis depends on a combination of serology and molecular biology, making it challenging to establish a definitive field test. The aim of this study was to use recombinase technology for the field detection of this virus, thereby enabling a point-of-care test. Glycoprotein E (gE), one of the primary targets for detecting animal herpesviruses, was chosen for isothermal amplification with a specific recombinase kit. Several primer, time, and temperature combinations were tested. The optimal amplification conditions were 41°C for 25 minutes. This combination proved effective for amplifying the gE fragment (226 bp), even when the template DNA was diluted 100-fold. These parameters were subsequently applied in the point-of-care assay, which used a lateral flow detection device and produced comparable results, with a limit of detection ranging from 5×10^2 to 10^1 viral copies. The point-of-care test was compared to a real-time PCR method reported in the literature for field samples with known positivity/negativity. There was perfect agreement with negative samples (34/34), and 18 of 26 positives were accurately diagnosed. The assay identified EHV-1 DNA in 11/12 highly positive samples (with a low Ct in real-time PCR) and 7/14 weakly positive samples (Ct more than 26). The overall diagnostic specificity was 100%, and the sensitivity was 69.2%, which increased to 91.7% when highly positive samples were used. This work represents the first application of this technology for EHV-1 detection. Although viral load affects accuracy, this device has the potential to be used in the field, as it requires no specialized equipment and yields results rapidly.

Keywords: Diagnosis, Equine herpesvirus, EHV-1, Herpesvirus, Recombinase, RPA

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Introduction

Varicellovirus equidalpha1, historically known as equid herpesvirus-1 (EHV-1), is one of the most common and impactful viruses affecting horses (Hussey, 2012). This virus, which belongs to the genus Varicellovirus and the subfamily *Alphaherpesvirinae*, is a significant concern because, like other varicelloviruses, it can cause respiratory disorders, abortion, and neurologic disease (which can be fatal) (Patel and Heldens, 2005). This disease is highly contagious because the virus can be transmitted via aerosols, contact with infected animals, and fomites (Oladunni et al., 2019). Latency reactivations after an initial infection play a significant role in the persistence of infection in horse populations. In fact, EHV-1 can remain latent for life in nervous system ganglia (e.g., the trigeminal ganglia) and in lymphoreticular tissues linked to the respiratory system, resulting in minimal viral gene expression and immune evasion (Laval et al., 2021). The reactivation of the virus and its spread via biological fluids can occur during stressful

events, particularly after parturition, lactation, environmental change, transport, or corticosteroid administration (Laval et al., 2021). The principal effects of EHV-1 on the equine industry are abortions in pregnant mares during the third trimester of gestation and disturbances to training schedules caused by infected horses with respiratory symptoms and fever, resulting in impaired athletic performance (Oladunni et al., 2019; Soboll-Hussey et al., 2024). In addition, outbreaks of a fatal type of EHV-1, herpesviral myeloencephalopathy (EHM), have increased in frequency (Hussey and Giessler, 2022; Kubacki et al., 2021; Pusterla et al., 2023). This outcome was first observed during a competition in Valencia (Spain) and was associated with amino acid substitution (asparagine to aspartic acid at position 2254) (Pusterla et al., 2009; Vereecke et al., 2021). For all of the reasons indicated above, it is necessary to accurately identify this infection to ensure the isolation of sick animals (as well as their treatment) and the interruption of the transmission cycle.

Successful identification of EHV-1 infection depends on several factors, including the timing of sample collection, selection of an appropriate sample based on clinical manifestations, use of relevant diagnostic assays, and careful evaluation and interpretation of laboratory results (Lunn et al., 2024). Laboratory diagnosis of EHV-1 relies on a combination of serology and molecular diagnostics. Several traditional serologic and virus isolation assays have been described (Khan et al., 2025). Although these tests are useful for identifying past viral exposure and perform well (essential for detecting animals with potential latent infections), they have limitations that prevent rapid, reliable detection of EHV-1 infection (Balasuriya et al., 2015). Recently, a DIVA protocol based on gB/gE has also been proposed, similar to that used for eradication plans for pseudorabies virus and infectious bovine rhinotracheitis (Ferrara et al., 2024; Ferrara et al., 2024; Osterrieder et al., 2024). The advent of molecular techniques has revolutionized the diagnosis of infectious diseases in humans and animal species. Specifically, polymerase chain reaction (PCR)–based assays have enabled precise and rapid detection of nucleic acid in clinical specimens, compared with traditional methods that detect the agent or antigen, or agent-specific antibodies, in serum (Balasuriya et al., 2015). New molecular methods, especially real-time PCR, are highly useful for detecting and identifying EHV-1. Specific PCR and real-time PCR protocols can detect even low viral doses (Diallo et al., 2006; Elia et al., 2006; Tallmadge et al., 2025). Molecular methods are used on individual animals prior to movement and to confirm clinical status (healthy/ill). These methods, although offering good diagnostic performance (it has been found that the agreement between PCR and the gold standard viral isolation is between 85 and 90 percent), lack practicality, as they require equipped laboratories, specific technical expertise, and several days to issue a report (Afify et al., 2024; Balasuriya et al., 2015). Because of these characteristics, they are not suitable for rapid field testing. Veterinarians working in horse practice must recognize the benefits and drawbacks of different nature tests, as well as their interpretations, to deliver the best possible treatment to their patients (Lunn et al., 2024).

In recent years, several PCR surrogates have been developed to enable field tests with “rudimentary” instruments. Recombinase polymerase amplification (RPA) is an isothermal nucleic acid amplification technology that has been shown to be a rapid, specific, sensitive, and cost-effective method for detecting pathogens (Li et al., 2019). RPA comprises bacterial enzymes such as strand-displacing DNA polymerase, recombinase, and single-strand binding protein. Thanks to the use of long oligonucleotides and probes, recombinase binds specific primers to template DNA for extension, followed by a polymerase for amplification/extension at isothermal temperature (Yan et al., 2014). The single-strand binding protein binds to displaced DNA strands and prevents primers from being displaced. Another advantage of this method is that, by labeling a primer probe with FAM conjoined with the opposing amplification primer, it is possible to read the amplification result with a simple lateral flow test. RPA does not require a thermal cycler because it allows amplification at a low, constant temperature (37-42°C) in 15-30 minutes using a simple water bath or heating block (Li et al., 2019). This next-generation amplification method has been successfully applied to a number of viral and bacterial pathogens, both in humans and in veterinary medicine. To date, it has received minimal attention for horse infections compared to other animal species, having only been examined for Hendra virus, *Streptococcus* spp., *Theileria equi*, and other pathogens (Knox and

Beddoe, 2021).

The purpose of this study was to evaluate the applicability of the RPA technique for EHV-1 detection and develop a lateral flow dipstick assay useful for field diagnosis.

Material and methods

Sequence choice and primer design

Two couples of primers were designed on the partial sequences of EHV-1 strain Kentucky D glycoprotein E (GenBank: AB279611.1) following the recommendations for recombinase amplification: amplicons of a maximum of 500 bp (226 bp in the current assay), length of primers (30-35 nucleotides), avoiding stretches of guanine bases in the first 5 nucleotides of the 5' primer end, and ensuring a GC content in the range of 30-70%. The primers used are listed in Table 1. Each primer combination was used in the RPA assay with the RAA Nucleic Acid Amplification Kit (Xpedite Diagnostics, Hallbergmoos, Germany) according to the manufacturer's instructions. Each reaction was placed in a specific tube containing 5 µL of DNA (extracted from 100 µL of the EHV-1 strain Kentucky D viral suspension, previously titrated at 106 with the Reed and Muench method) (Montagnaro et al., 2019). The reaction tubes were first incubated for 30 minutes at 41°C. After establishing the best primer combination, the assay was evaluated at different temperatures (25, 30, 37, 39, and 41°C), times (10, 15, 20, 25 minutes), and viral serial dilutions (EHV-1 DNA diluted in molecular biology water). Each sample was purified using the QIAamp® DNA Mini Kit (Qiagen, Hilden, Germany) and run on an agarose gel to visualize the results.

Optimization of the RAA-LFD assay

Once the working conditions were optimized, additional primers were designed to enable detection of the reaction by a lateral flow device (Table 1). In particular, a probe was also designed following the manufacturer's recommendations (length of 46-52 nucleotides, and the 3' end must be blocked by a suitable modification like C3 spacer to avoid primer extension). Moreover, both the fluorophore and the quencher were attached as internal modifications to the oligonucleotide. The fluorophore was attached to a base at least 30 nucleotides away from the 5' end. The quencher was attached to a base at least 15 nucleotides away from the 3' end and should be located 2-4 nucleotides downstream (on the 3' side) of the fluorophore. One of the bases between the fluorophore and quencher was replaced by a basic nucleotide (tetrahydrofuran, THF). The same forward primers and a modified reverse primer (with a biotin moiety at the 5' end) were used to optimize the RAA-LFD assay. Each reaction was composed of 25 uL Buffer V, 2.5 µL Magnesium acetate, forward and reverse primers (10 µM) 2 µL, probe (10 µM) 0.6 µL, water 13.5 µL, and 5 µL of DNA. Once amplification was complete, the tubes were carefully opened, and 50 µL of the amplification reaction was transferred to a 1.5mL microcentrifuge tube containing 450 µL 1× Phosphate Buffered Saline (PBS). The fluorophore (FAM) was aligned with the antibody combination used on the lateral-flow strip (Nucleic Acid Lateral Flow Immunoassay [NALFIA], Abingdon Health, York, United Kingdom). Amplification products were detected by adding the diluted amplification reaction to an appropriate lateral-flow strip (Abingdon Health, York, United Kingdom). Different dilutions of template DNA (extracted from the virus), times (30, 25, 20, 15, 10, 5, 2 minutes), and temperatures (41, 39, 37, 30, 25°C) were used. Moreover, for the calculation of Limit of Detection (LoD), the RAA-LFD assay was compared to a real-time PCR using known amounts of DNA (copy number). For this purpose, the gE fragment was amplified and directly cloned into a plasmid using TOPO® TA Cloning® Kits (Thermo Fisher Scientific, Waltham, Massachusetts, United States). The cloned product was purified using the QIAprep Spin Miniprep Kit and quantified by a Nanodrop at 260 nm (Thermo Fisher Scientific, Waltham, Massachusetts, United States). The DNA copy number was calculated by the equation: DNA copy number= $(M \times 6.02 \times 10^{23} \times 10^{-9})/(n \times 660)$, M: molecular weight, n: plasmid concentration.

Diagnostic performances using clinical samples and a column-free DNA extraction kit

A total of 56 horses with known clinical status were sampled (a double nasopharyngeal swab was collected). The samples are part of a routine screening sampling carried out on horses with respiratory problems. One swab was extracted with a commercial kit (DNeasy Blood & Tissue Kits, Qiagen, Hilden, Germany), and the DNA was used as template in a real-time PCR protocol described in the literature using a commercial probe mix (iTaq Universal Probes Supermix,

Biorad, Milan, Italy) (Elia et al., 2006). The second swab was extracted using a column-free DNA extraction kit (SwiftX™ Swabs, Xpedite, Hallbergmoos, Germany). Briefly, each swab was vortexed for 5 seconds in 500 µL activated Component E and incubated for 15 minutes at 90°C. Extracted DNA was used as a template for the RAA-LFD assay. The results were compared using real-time PCR as the reference test, and the Kappa (K) statistic was calculated. Values of K < 0.2 were considered "poor", 0.21–0.4 "fair", 0.41–0.6 "moderate", 0.61–0.8 "good", and 0.81–1.00 "very good" (Ferrara et al., 2023). Furthermore, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) values were calculated.

Table 1: Primers used in this work: Primers A and C were used to optimize the RPA assay with agarose gel reading.

Name	Sequence (5´-3´)	Position
Primer A	ACCCTCCACGTTTCCATCGACGGAATGATG	448-478
Primer B	GTTTACACCCTCCACGTTTCCATCGACGGA	442-472
Primer C	GTGACTCCAGGTTCACCGACAGCTCGAAAG	644-674
Primer D	GATATGTGACTCCAGGTTCACCGACAGCTC	649-679
Forward (def.)	ACCCTCCACGTTTCCATCGACGGAATGATG	448-478
Reverse (def.)	[Biotin]-GTGACTCCAGGTTCACCGACAGCTCGAAAG	644-674
Probe (def.)	[FAM]-CCGTTCCTCAGGTTCCC GTAAAGACCCACACGGAT [dSpacer]TT GTGGTG[C3Spacer]	563-607

Forward and reverse (def.) primers and the probe (def.) were used to optimize the RAA-LFD assay and to test the clinical samples. In addition to the nucleotide sequence, the position in the whole sequence of gE is present (1653 bp).

Results

Different primers (two forward and two reverse) were tested to amplify an approximately 226-bp fragment of EHV-1 gE by recombinase assay. The optimal combination was AC, as revealed by the agarose gel electrophoresis after purification. The combination AC showed the highest quantities of amplified product (Figure 1). Recombinase amplification was subsequently optimized with different combinations of time, temperature, and viral DNA dilution. The amplicon was clearly visible with incubation at 37-41°C, but it was very faint when the reaction was kept at 30°C (Figure 1). The same trend was observed for reaction time: the amplicon was clearly visible after 25 minutes of incubation, but it was less visible at 20 and 15 minutes (absent at 10 minutes). These conditions (25 minutes at 41°C) were used to test different dilutions of viral DNA. The 226 bp amplicon was clearly visible in samples diluted up to 100-fold, faintly visible at 1000-fold, and absent at 10,000-fold and 100,000-fold. Subsequently, the same conditions were maintained to test the recombinase's suitability for lateral-flow detection with specific dipsticks (Figure 2). The previously obtained results were confirmed with a lateral-flow assay. No significant differences in reaction rate were observed over the 41-37°C range, whereas lower temperatures significantly reduced the reaction's ability to amplify template DNA. The test detected EHV-1 DNA when incubated for 25-30 minutes. The results were also confirmed by dilution of viral DNA, with a weak band observed even at a 1000-fold dilution (Figure 2). The results were further investigated by comparing the RAA-LFD assay with real-time qPCR, using known amounts of template DNA. The LoD of the RAA-LFD ranged from 5×10^2 to 10^1 viral copies. Field sample testing provided indications regarding the device's potential use as a point-of-care test (Tables 2 and 3).

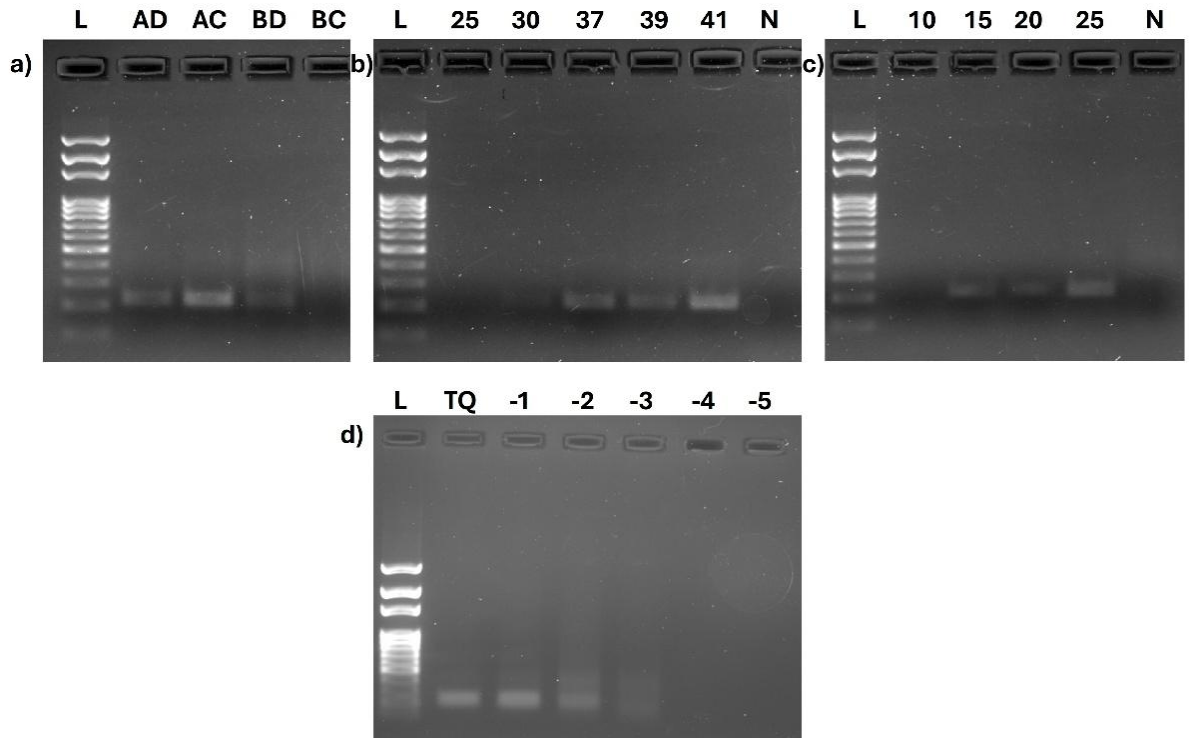


Figure 1: Optimization of RPA using different primers (a) and parameters [temperature (b), time (c), viral DNA dilution (d)]. The PCR product (236 bp) was easily observed with the AC primer combination at 41°C for 25 minutes.

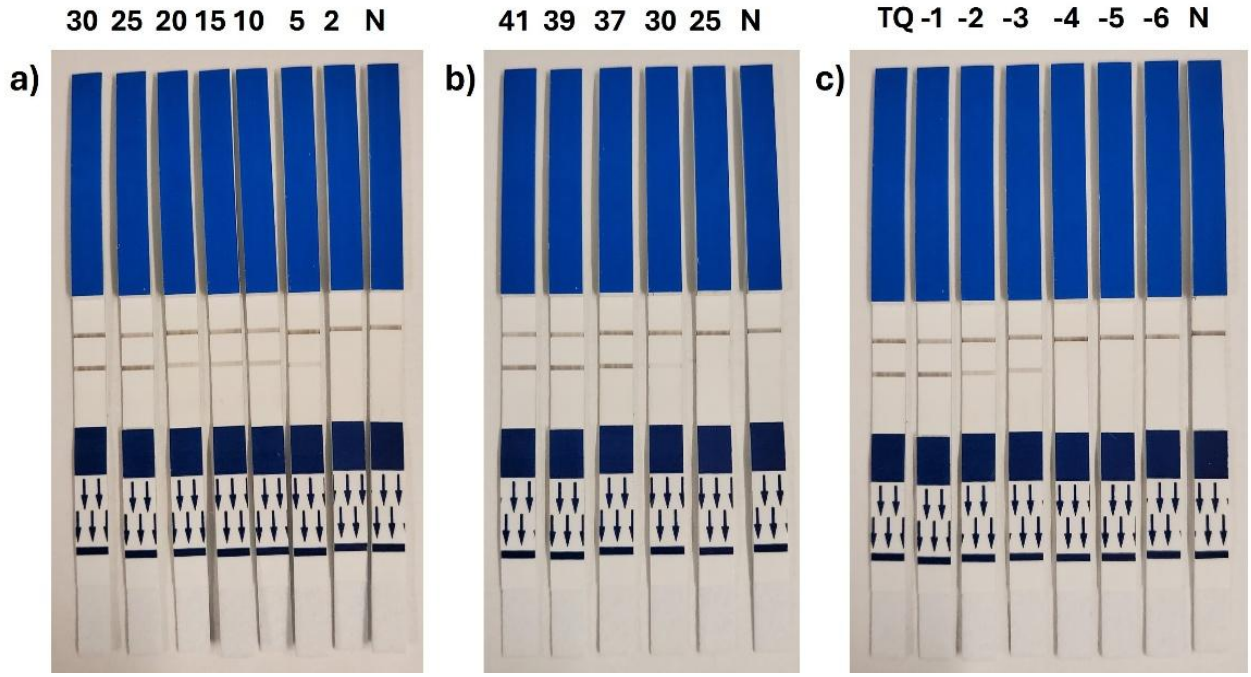
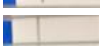
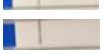


Figure 2: Optimization of the RAA-LFD assay using different times (a), temperatures (b), and viral DNA dilutions (c). The reaction was easily readable in lateral flow using the 25-30 minutes time interval (a) and the 37-41°C temperature range (b). The reaction was evident with viral DNA diluted up to 10-fold, but less visible at dilutions up to 1000-fold (c).

Specifically, 34 of 34 negative animals were correctly identified (Table 2). Of the 26 real-time positives, only 18 were correctly identified, including 11/12 strong positives and 7/14 weak positives (Table 3). The results showed 86.7% agreement with the gold standard and a Cohen's k of 0.72, which is considered "good" (Table 4).

Table 2: Diagnostic performances of the RAA-LFD assay using clinical samples (negative in real-time PCR).

ID	Real-time PCR	Ct	RAA-LFD	C / T
4	Negative	/	Negative	
5	Negative	/	Negative	
6	Negative	/	Negative	
10	Negative	/	Negative	
11	Negative	/	Negative	
12	Negative	/	Negative	
13	Negative	/	Negative	
14	Negative	/	Negative	
15	Negative	/	Negative	
16	Negative	/	Negative	
17	Negative	/	Negative	
20	Negative	/	Negative	
23	Negative	/	Negative	
24	Negative	/	Negative	
26	Negative	/	Negative	
28	Negative	/	Negative	
30	Negative	/	Negative	
31	Negative	/	Negative	
34	Negative	/	Negative	
35	Negative	/	Negative	
39	Negative	/	Negative	
42	Negative	/	Negative	
43	Negative	/	Negative	
45	Negative	/	Negative	
46	Negative	/	Negative	
47	Negative	/	Negative	
48	Negative	/	Negative	
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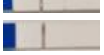
54	Negative	/	Negative	
55	Negative	/	Negative	
57	Negative	/	Negative	
58	Negative	/	Negative	
59	Negative	/	Negative	
60	Negative	/	Negative	

ID= Laboratory identifier

Ct= Cycle threshold value

RAA-LFD= Recombinase-Aided Amplification-Lateral Flow Dipstick

C / T= Control / Test

These findings were confirmed by assessing sensitivity, specificity, PPV, and NPV. The RAA-LFP demonstrated a sensitivity of around 70% and a specificity of 100%. Sensitivity improved to 91.7% when the results of the strongly positive samples were compared but decreased to 50% when only the weakly positive results were included (Table 5). PPV and NPV values showed the same trend.

Table 3: Diagnostic performances of the RAA-LFD assay using clinical samples (positive in real-time PCR).

ID	Real-time PCR	Ct	RAA-LFD	C / T
32	Positive	Low (19.3)	Positive	
33	Positive	Low (21.7)	Positive	
18	Positive	Low (22.1)	Positive	
22	Positive	Low (22.5)	Positive	
52	Positive	Low (19.8)	Positive	
1	Positive	Low (24.9)	Positive	
7	Positive	Low (23.3)	Positive	
9	Positive	Low (24.5)	Positive	
21	Positive	Low (24.1)	Positive	
29	Positive	Low (21.4)	Negative	
41	Positive	Low (20.9)	Positive	
2	Positive	High (32.3)	Positive	
3	Positive	High (34.7)	Negative	
8	Positive	High (31.2)	Negative	
53	Positive	High (26.1)	Positive	
56	Positive	High (26.4)	Positive	
19	Positive	High (32.5)	Negative	
21	Positive	High (33.2)	Negative	
25	Positive	High (29.9)	Positive	
27	Positive	High (30.9)	Positive	
36	Positive	High (29.7)	Positive	
37	Positive	High (28.3)	Positive	
38	Positive	High (34.2)	Negative	
40	Positive	High (33.2)	Negative	
44	Positive	High (33.5)	Negative	

ID= Laboratory identifier, Ct= Cycle threshold value, RAA-LFD= Recombinase-Aided Amplification-Lateral Flow Dipstick, C/T= Control/Test

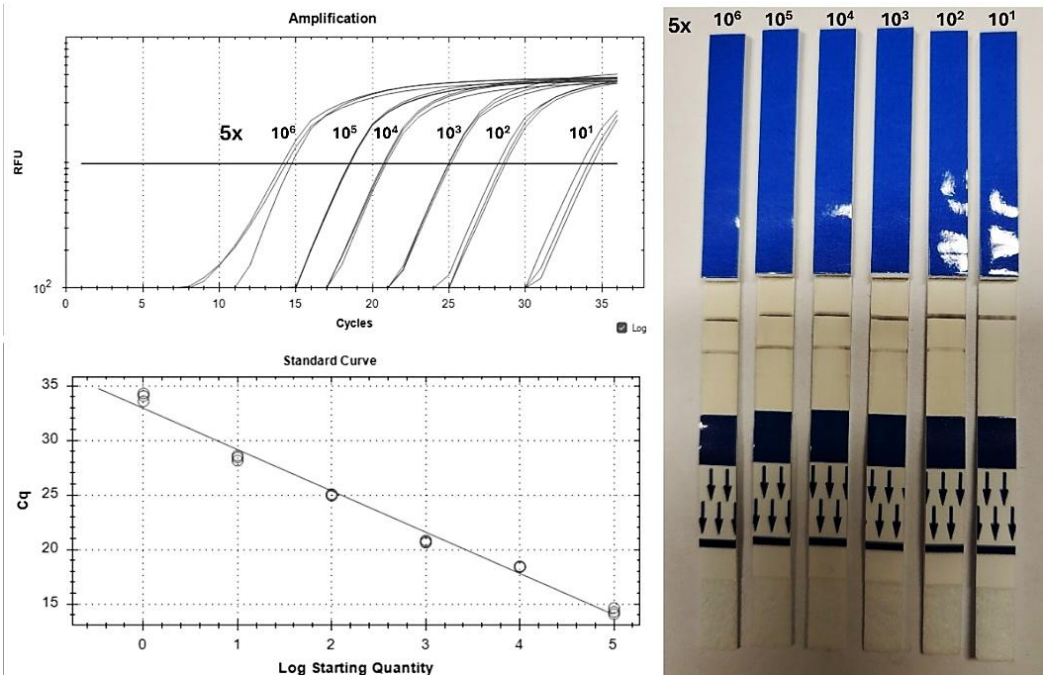


Figure 3: Optimization of the RAA-LFD assay using different template amounts.

Table 4: Comparison of results obtained with RAA-LFD and real-time PCR (gold standard).

Outcome	n	Agreement	Cohen κ (95% CI)
Positive	18/26		
Highly positive	11/12		
Weakly positive	7/14		
Negative	34/34		
Overall	52/60	86.7	0.72 (0.54–0.89)

CI= confidence interval.

Table 5: Diagnostic performance (sensitivity, specificity, positive predictive value, negative predictive value) obtained with RAA-LFD and real-time PCR (gold standard)**.**

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Overall	69.2 (51.5–87)	100 (100–100)	100 (100–100)	81 (69.1–92.8)
Highly positive	91.7 (76–100)	100 (100–100)	100 (100–100)	97.1 (91.6–100)
Weakly positive	50 (23.8–76.2)	100 (100–100)	100 (100–100)	82.9 (71.4–94.4)

Discussion

Diagnostic confirmation of EHV-1 remains a topic of discussion, and numerous efforts are underway to improve or replace the diagnostic tests currently used for field samples. As also underlined by the latest consensus of the ACVIM (American College of Veterinary Internal Medicine), real-time qPCR is the choice test in both respiratory secretions and blood samples because of its high diagnostic performance, although it consists of a time-consuming assay that does not properly represent an on-field assay (Lunn et al., 2024).

In the present study, recombinase technology was applied for the first time to detect EHV-1. The test demonstrated good diagnostic performance (86.7% agreement) compared with real-time PCR on field samples. The best results were obtained using strongly positive samples (sensitivity of 91.7%). This test had the advantages of being rapid (30 minutes), inexpensive, easy to perform and interpret (due to the lateral flow detection system), and reproducible under field conditions. Another advantage of the technology developed in this work is the elimination of DNA extraction, a significant bottleneck for field applications in molecular biology. In contrast, column-free systems require just a thermostat. Furthermore, since the test focused on gE and gE-deleted vaccines, which are increasingly widespread, the circulation of any vaccine strains should not affect the results, thereby avoiding cross-reactions (Osterrieder et al., 2024).

In recent years, other attempts have been made to produce new field tests based on different technologies with encouraging results (Knox and Beddoe, 2021). For example, a gold nanoparticle biosensor for ultrasensitive detection and genotype identification has recently been designed (Ghoniem et al., 2023). This device demonstrated a very low limit of detection (one copy of viral DNA) and analytical and diagnostic sensitivities higher than those of real-time PCR (Ghoniem et al., 2023).

The enzyme-linked aptamer sandwich assay (ELASA) has been proposed as an antigenic test that eliminates the DNA extraction and gel electrophoresis steps (Davoudi et al., 2026). This test was able to detect 10 viral particles per milliliter, compared to the 1000 viral particles per milliliter required by conventional PCR

The detection of EHV-1 was also evaluated with real-time fluorometric and end-point colorimetric isothermal assays (LAMP) (Jelocnik et al., 2021; Nemoto et al., 2010). The comparison with the gold standard revealed very good diagnostic performances (98.86% agreement and 91.43% agreement, respectively, for rtLAMP and LAMP). Compared with a commercially validated qPCR assay, the EHV-1 rtLAMP assay demonstrated 86.96% sensitivity and 100% specificity, with an overall agreement of 91.43% (Jelocnik et al., 2021).

These findings highlight the strong potential of these assays as cost-effective, fast, user-friendly, and on-field point-of-care diagnostics, particularly in low-resource settings. To reduce sample testing turnaround time, several stall-side molecular EHV-1 testing platforms have been described, and new technologies may be relevant to these platforms. Although rapid testing is useful for treating acute cases, new point-of-care EHV-1 diagnostics must be well-

validated and demonstrate acceptable agreement with gold-standard tests, such as qPCR and virus-isolation assays. In practice, these tests are compared with gold standards using clinical samples with high viral loads, thereby neglecting borderline cases and relying on a small number of samples, generally fewer than 100 (Lunn et al., 2024).

The device setup for detecting EHV-1 DNA discussed in this study is not the only application of this technology in horses. RPA-LFD was used for the rapid detection of *Burkholderia mallei*, with high sensitivity and specificity (Saxena et al., 2019). Furthermore, recent studies have obtained similar results with RPA combined with CRISPR/Cas-LFD for the detection of *Theileria equi* and *Streptococcus equi* (Alsultan et al., 2025; Lei et al., 2020; Zu et al., 2024, 2025).

Similarly, similar detection systems have been developed for most animal and human herpesviruses, like feline herpesvirus type-1 (FHV-1), bovine herpesvirus type-1 (BHV-1), pseudorabies virus (PRV), and others (Hou et al., 2017; Liu et al., 2019; Wang et al., 2017; Yang et al., 2017).

These diagnostic tools are not without limitations. In addition to those already highlighted above, there are concerns about the assay's true sensitivity and standardization. Regarding characterizing a larger number of samples for validation, there are also concerns about the assay's true sensitivity and standardization. For example, in the present study, it was found that samples with lower viral titers (Ct between 31.2 and 34.7) did not react in the RAA-LFD assay. A better characterization would include samples from experimental infections at different dpi (days post-infection) to determine exactly when and how the test would be useful for detecting the virus. Further investigations would include the possible differentiation of classical strains from neurotropic ones with double-probe instruments working in duplex amplification or the application of the method to different matrices, such as blood or abortion products. Furthermore, for accurate validation, it would be necessary to evaluate the reproducibility and repeatability of the RAA-LFD assay to assess inter- and intra-assay variability.

Another aspect to be verified is the usefulness of the double sampling (two-stage) approach, as suggested by ACVIM (Oladunni et al., 2019). In fact, as stated by the latest consensus, when horses are collected on multiple days in a row, both blood and nasal secretions have equivalent sensitivity for detecting EHV-1 using qPCR. When horses are sampled only once (for example, during outbreaks), nasal secretions are more likely to detect EHV-1 in horses with fever and signs of respiratory disease, as well as in horses with suspected neurotropic strains (Oladunni et al., 2019).

EHV-1 represents a major health problem in horses, and improving diagnostic screening tests is of paramount importance. Further efforts are needed to ensure that these devices can effectively replace currently available tests.

Conclusions

Herpesvirus infections in horses, like those affecting other domestic animals, have a considerable impact and pose a significant problem. This work developed a simple, rapid, and cost-effective point-of-care test based on recombinase technology that can be read with lateral-flow dipsticks. Despite high diagnostic performance compared with the gold-standard test (real-time PCR), additional research is needed to confirm these features with a larger sample set and to determine whether these new technologies will replace existing approaches.

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Authors' contributions: All authors contributed equally to the work.

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