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Evaluating two approaches for using positive control in standardizing the avian influenza H5 reverse transcription recombinase polymerase amplification assay

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ABSTRACT

Highly pathogenic avian influenza H5N1 virus causes heavy losses in poultry farms worldwide. Molecular diagnostic techniques like RT-PCR and real-time RT-PCR are considered the gold standard for identification of H5 influenza viruses in clinical samples. These techniques are hampered by the need of well-equipped laboratories, large space requirement, and relatively long time-to-result. Recombinase polymerase amplification (RPA) assay represents an excellent alternative to PCR since it is more simple, rapid, economic, and portable. Reverse transcription RPA (RT-RPA) assay was recently developed for sensitive and specific detection of H5N1 virus in 6–10 min. To ensure the accuracy of the developed assay, two approaches for using a positive control were evaluated in this study. These approaches included: 1) all-in-one (internal positive control; IPC), 2) two-tubes-per-one-sample (external positive control; EPC). Sigma virus (SIGV) RNA and turkey mitochondrial DNA were tested as positive controls in both approaches. For all-in-one approach, both targets (H5 and IPC) were strongly inhibited. In contrast, very good amplification signals were obtained for the two types of EPC with no effect on the analytical sensitivity and specificity of H5 RT-RPA assay in two-tubes-per-one-sample approach. The performance of EPC-based H5 RT-RPA was further validated using 13 tracheal swabs. The results were compared to real-time RT-PCR and proved superior specificity in detecting H5N1 but not H5N8 viruses. Inclusion of EPC did not affect the aptitude of both assays in terms of sensitivity, specificity and reproducibility. In conclusion, the two-tubes-per-one-sample approach was more reliable to control the false negative results in H5 RT-RPA assay.

1. Introduction

Highly Pathogenic Avian Influenza (HPAI) is an acute contagious disease that affects domestic poultry causing severe economic losses. Infrequently, the disease can be directly transmitted from infected birds to humans and results in severe public health threats [1]. The HPAI caused by H5N1 avian influenza virus was originally developed in China in 1996, and consequently spread towards Europe and Africa crossing central and western Asia. The strains descended from this outbreak continued to spread in more than sixty countries, with emergence of several distinct phylogenetic clades [2,3].

HPAI viruses belong to genus *alphainfluenzavirus* in the family *Orthomyxoviridae*. Eight segments of negative sense single stranded RNA are complexed with the nucleoprotein and enclosed with the viral polymerase subunits in a lipid envelope. Two major glycoproteins that determine the viral antigenicity are projecting from the surface of the lipid envelope; hemagglutinin (HA) and neuraminidase (NA) [4].

Molecular techniques are the gold standard for routine identification of H5 influenza viruses in endemic countries [5]. Methods like reverse transcription PCR (RT-PCR) and quantitative RT-PCR (qRT-PCR) are demanding at the levels of infrastructure, containment and expertise. Time-to-result is relatively long [6]. Recombinase polymerase amplification (RPA) is a recently developed isothermal amplification technique that employed phage recombinase and co-factor to

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eliminate the need for denaturation and annealing steps in traditional PCR [7]. This technique combined robustness with minimum space requirement and the ability to be used at point-of-need [8]. RPA and RT-RPA were utilized for rapid detection of many viruses of veterinary importance [6,9–13]. RT-RPA was recently described for diagnosis of H5N1 virus in clinical samples in just 6–10 min. The developed assay has proven high specificity, sensitivity, reproducibility, and robustness comparable to qRT-PCR. It was able to detect as low as one copy of *in vitro* transcribed RNA Standard [11].

RPA reaction validation is a critical requirement to ensure the quality and accuracy of the test. False negative results may arise from inhibitory substances, poor performance of the enzymes, and/or incorrect RPA mixture. The use of positive controls is a common strategy to avoid false negative results in PCR (and certainly in RPA). With the aim to improve the performance of the recently developed H5 RT-RPA in field applications, positive controls were tested and compared in different settings. Sigma virus (SIGV) RNA and turkey mitochondrial DNA were evaluated using two approaches: All-in-one approach (internal positive control; IPC), and two-tube-per-sample approach (external positive control; EPC).

2. Materials and methods

2.1. Quantitative molecular standards, RPA primers and exo-probes

Influenza A H5 molecular RNA standard was synthesized by amplification of a 970 bp fragment of HA2 gene of the Egyptian H5N1 reference strain A/chicken/Egypt/1273CA/2012 using RT-PCR. The amplified fragment was ligated into pCRII vector by TA Cloning and the RNA standard was *in vitro* transcribed and quantified [11]. The quantitative RNA standard of SIGV was generated according to the method developed by Weidmann et al. [14]. Turkey DNA standard was synthesized by GeneArt (Thermofisher, Regensburg, Germany) as a short string of 300 bp of conserved *Meleagris gallopavo* mitochondrial DNA sequence. The primers and exo-probes used for detection of Influenza A H5N1 and SIGV were prepared according to Yehia et al. [11] and Euler et al. [8], respectively. Few modifications were introduced to the exo-probe used for detection of SIGV to adapt TAMRA and/or ROX channel detection (TIB Molbiol GmbH, Berlin, Germany). All primers and exo-probes (Table 1) were synthesized to fit with the requirements of Twist Amp™ exo RT kit (Twist Dx, Cambridge, UK).

2.2. RPA reaction setup and result interpretation

RPA was performed in 50 μl reaction volumes using TwistAmp™ Exo-RT kit for H5N1 influenza virus and SIGV, and TwistAmp™ Exo kit for turkey DNA (TwistDx, Cambridge, UK). The reaction mixture contained 420 nM of each RPA primer and 120 nM of the exo-probe, 14 mM of magnesium acetate, and TwistAmp rehydration buffer. One microliter of the DNA/RNA template was added to the mixture after dispensing into the reaction tubes containing dried enzyme pellets, and the reaction volume was completed by nuclease-free water. The tubes were briefly centrifuged and placed directly in ESEQuant Tube Scanner™ (Qiagen, Lake Constance, Germany) at 42 °C for 15 min. Fluorescence measurement was performed each 20 s in the FAM channel for H5N1, TAMRA or ROX channel for SIGV, and ROX channel for Turkey DNA. Specific amplification was verified by the tubescanner studio by increase in the fluorescence intensity in 1st derivative analysis and over time.

2.3. Optimization of IPC-based H5 RT-RPA assay (all-in-one approach)

The effect of including different types of non-competitive IPC on the analytical sensitivity of the previously developed H5 RT-RPA assay was evaluated in a single-tube setting. A 10-fold dilution series of the *in vitro* transcribed H5 RNA standard was prepared – in triplicates – from 10^6 to 10^0 copies/μl. The sensitivity limit was determined as the highest dilution that showed exponential amplification above the threshold of the negative control within 6–10 min. Similar dilution sets of the quantitative standards of SIGV and turkey DNA were tested in parallel for determination of the suitable concentrations for use as IPCs; highest dilutions that generated amplification curves after 6–7 min. Duplex reactions that include both H5 and IPC primers and exo-probes were prepared in groups. Each group received constant concentration of the IPC standard and a dilution series of H5 RNA standard. All groups were tested in triplicates including separate no-template-control tubes.

2.4. Optimization of EPC-based H5 RT-RPA assay (two-tube-per-sample approach)

To avoid potential assay inhibition, two separate monoplex reactions; one for H5 and the second for the positive control, were tested in parallel. The 10-fold dilution series of H5 RNA standard and the previously determined constant dilution of each positive control were assayed in single RPA runs. No-template control was included in all assays. The performance of each EPC in this approach was analyzed in triplicates within and among assays.

2.5. Validation of assay performance on clinical samples

Thirteen tracheal swabs were collected from chicken showing indicative clinical signs for infection with HPAI virus from different Egyptian governorates between 2016 and 2018. Five samples were collected from specific-pathogen-free chicken and served as negative controls. Viral RNA was isolated using QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. All samples were concurrently tested using the gold standard real-time RT-PCR [11] and the EPC-based H5 RT-RPA assay. The performance of both assays in detection of H5N1 virus and positive control was compared and analyzed in terms of sensitivity, specificity, and

Table 1
Sequence of RPA primers and exo-probes.

Name	Sequence (5′- 3′)
H5 RPA-FP	TAACGGTTGTTTCGAGTTCTATCACAGATG
H5 RPA-RP	ACTTATTTCCTCTCTTTTTAATCTTGCTTC
H5 Exo-probe	GTATGGAAAGTGTAAGAAACGGAACGTA (BHQ1-dT) THF(FAM-dT)TACCCGCARTATTC-PH
SIGV RPA-FP	TGACCATCCTAACTCTGTGACATTCCAAGT
SIGV RPA-RP	GTTGACAGTGAGCTCTTGAATCTCTGGGTT
SIGV RPA-P-TAMRA	ACTGATTTCCCTCCGTGTCCTCCCGGTACCAC-(BHQ2-dT) THF(TAMRA-dT)-CCAAACTGCCGTTGTG-PH
SIGV RPA-P-ROX	ACTGATTTCCCTCCGTGTCCTCCCGGTACCAC-(BHQ2-dT) THF(ROX-dT)-CCAAACTGCCGTTGTG-PH
Turkey RPA-FP	CTAATAACAACAACCATATTCTTATCATTAACCC
Turkey RPA-RP	GCCGGCTAGAGATAGGAGTGCAAGTATTATAG
Turkey RPA-P-ROX	ATCATTAACCCAGATCAAAGTCCTGAAAC-(BHQ2-DT)THF (ROX-DT)CAACAATACTCATC-PH

FP: forward primer; RP: reverse primer; P: probe, BHQ-dT: thymidine nucleotide carrying Blackhole quencher, THF: tetrahydrofuran, FAM-dT: thymidine nucleotide carrying Fluorescein, PH: phosphate group.

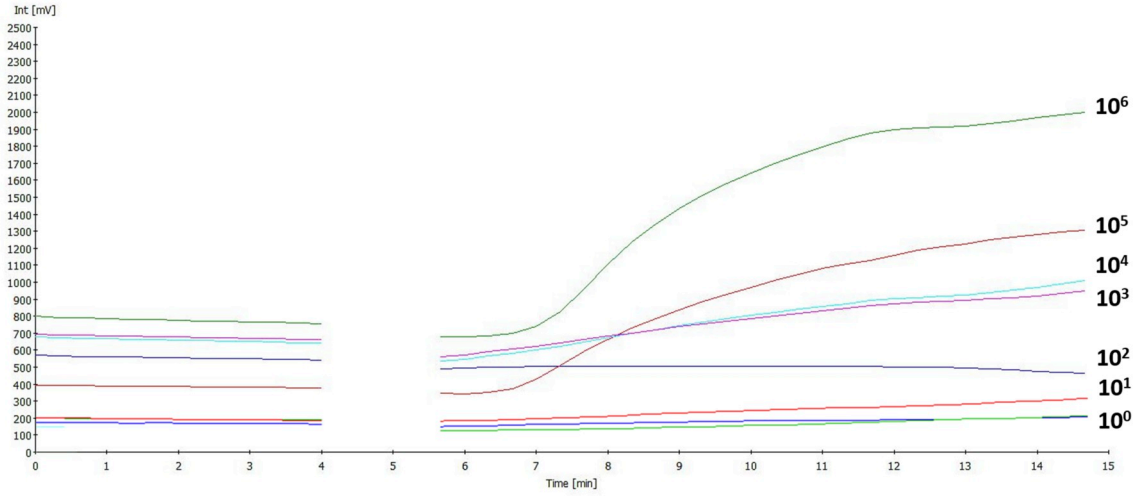

Fig. 1. Analytical sensitivity of H5 RT-RPA assay. A graph generated by ESEQuant Tube Scanner™ software showing fluorescence development overtime of the *in vitro* transcribed H5 RNA molecular standard (dilution range of 10^6 to 10^0 copies/reaction). The concentration of the RNA standard is indicated at the right side of the corresponding curve.

reproducibility.

3. Results and discussion

3.1. Analytical sensitivity of single-target (monoplex) RPA assays

The different RPA assays targeting Influenza A H5N1 virus, SIGV, and turkey DNA were independently evaluated for their performance before combined detection of multiple targets was examined. A 10-fold dilution series (10^6 to 10^0 copies/μl) of each standard was prepared in triplicates and was tested using the relevant primers and exoprobe. The analytical sensitivity of H5 RT-RPA assay ranged from 10^2-10^3 molecules per reaction (Fig. 1), whereas the detection limit in RPA assays used for detection of SIGV (either using ROX- or TAMRA-labelled exoprobes) and turkey DNA was 10^3 molecules per reaction (Table 2).

In an earlier report, the H5 RT-RPA assay was developed and detected down to a single-copy of the *in vitro* transcribed RNA standard [11]. Likewise, the detection limit of SIGV RNA standard was previously reported to range from 10^1-10^2 copies per reaction [8]. This indicates that the analytical sensitivity of the current assays are 1–3 logs lower than those described before. Since there was no specific difference between current and previous assays in terms of design, instrumentation, and personnel, it may be expected that the lower sensitivity of the current assays is regarded to: i) the use of different reagent batches; ii) the deterioration of primer and exoprobe aliquots by repeated freezing and thawing, iii) the gradual decrease in instrument aptitude over time. This observation is not surprising since even in a well established quatitative real time PCR systems, fine changes in the experimental procedures or reagent sources can drop the analytical sensitivity up to 6 folds [15]. However, validation of all reagents and instrumentation are deemed essential in reproducibility and repeatability of quatitative molecular biology experimentations including RPA [16,17].

3.2. Performance of IPC-based H5 RT-RPA (duplex) assays

Exogenous IPC is often added to the amplification reactions to ensure quality control of the results and to avoid false negatives [18]. Non-competitive IPC; that is not homologous to the target(s), is mostly preferable for several reasons including: ease of design and synthesis, low capacity of inhibition of one or both reactions due to competition, and ability of using the same IPC in different assays simultaneously [19]. Two non-competitive IPCs were evaluated in the current study; 1) SIGV; an insect rhabdovirus, which is theoretically impossible to be present as a contaminant in chicken samples at any instance [20], 2) turkey mitochondrial DNA, which is characteristic for turkey cells and is completely different from similar sequences found in chicken tissues. Mitochondrial DNA is routinely used for tracing the history and evolution of domestic and wild turkeys [21]. Both types of IPC were optimized in RPA assays and their sensitivity limits were determined as

Table 2
Analytical sensitivity of RT-RPA assays used for optimization of All-in-one approach.

Concentration (Copies/μl)	Monoplex Reactions				Duplex Reactions		
	H5 STD FAM	SIGV STD TAMRA	SIGV STD ROX	Turkey DNA STD ROX	H5 STD FAM SIGV STD TAMRA[a]	H5 STD FAM SIGV STD ROX[a]	H5 STD FAM Turkey DNA STD ROX[a]
10^6	+	+	+	+	–	–	–
10^5	+	+	+	+	–	–	–
10^4	+	+	+	+	–	–	–
10^3	+	+[c]	+	+	–	–	–
10^2	±[b]	–	–	–	–	–	–
10^1	–	–	–	–	–	–	–
10^0	–	–	–	–	–	–	–

STD: Standard.
[a] A single concentration (10^4) of the CIPC was used in the reaction.
[b] Weak and inconsistent signal.
[c] Weak but consistent signal.

shown above. The concentration of 10^4 copies per reaction was chosen for all IPCs in optimization of duplex assays as it represents the least amount of DNA/RNA standard that yielded consistent amplification signals.

The performance of H5 RT-RPA assay in the presence of non-competitive IPC was evaluated but unfortunately no amplification signals were developed for both targets; H5 and IPC (Table 2). No variation in the results was observed by changing the type of IPC, the detection channel, and the concentration of the two targets.

RPA has proven superior performance in detecting different pathogens (bacteria, fungi, protozoa, viruses, and others) that infect a wide array of vertebrate, invertebrate and plant hosts [22]. However, optimizing multiplex RPA assays that are capable to detect multiple targets simultaneously or that include IPC was mostly ineffective. A possible explanation is that RPA conditions cannot tolerate the presence of a cocktail of nucleic acids and are strongly inhibited when background DNA exists in particular concentrations [23].

However, the ability to develop multiplex assays was only achieved when RPA was combined with other technologies that enabled: 1) immobilization of the primers on a solid surface (microfluidic chips) to combine the performance of RPA with the multiparameter analysis of microarray systems [24], 2) visual detection of the amplicons using lateral flow readouts [23,25,26]. However, the use of lateral flow reduced the analytical sensitivity of individual target molecules in the multiplex reaction [25], 3) simultaneous extraction, amplification, and fluorescent detection in a centrifugal chip [27], 4) product detection achieved using surface-enhanced Raman Scattering labelled nanotags; RPA-SERS [28]. Even though, in most cases, the targets detected by the multiplex RPA assays do not simultaneously exist in the clinical samples, and consequently the assay is performed as a monoplex reaction [25–27,29,30]. The use of multiplex RPA to simultaneously detect RNA targets is not available so far. Therefore, in the current protocol, we have evaluated a duplex assay that included a previously optimized RNA target (H5N1 influenza virus) and an IPC of either RNA (SIGV) or DNA (turkey mitochondrial DNA) nature. The failure of the reaction to generate positive signals under all conditions provides additional evidence that the available RPA conditions are demanding for use in multiplexing. It may require further improvements in the reaction-setup, instrumentation, and supporting technologies; yet maintaining the potential of RPA in simplicity, speed, and ability to use in point-of-need facilities.

3.3. Aptitude of the two-tube-per-sample as an alternative approach

The effect of co-amplification of H5 and EPC in two-dependent monoplex RPA reactions was analyzed for use as an alternative to the inefficient IPC-based RT-RPA assays. The different EPC tested (SIGV-ROX, SIGV-TAMRA, and turkey mitochondrial DNA-ROX) produced clear amplification signals after 6–7 min, while maintaining the analytical sensitivity of H5 RT-RPA assay. No significant intra-assay or inter-assay variation was determined between the replicates.

False negative results in amplification reactions is mostly attributed to three main factors: 1) failure of one or more of the reaction components, 2) existence of inhibitors, 3) instrument failure [31]. Although IPCs are routinely used to efficiently control the three elements in several amplification-based assays, their insufficiency in RPA may require shift to the use of EPCs. The use of EPC controls for successful instrument performance and integrity of the reaction mixture, but it does not exclude the presence of inhibitors in the reaction. Together with the ease of design and optimization of the reaction, the use of EPC is satisfactory right now and provides a good alternative to the IPC, with no need for combination of the RPA assay with sophisticated and complicated platforms/procedures.

3.4. Analysis of clinical samples

The performance of EPC-based H5 RT-RPA assay in field applications was validated using 13 tracheal swabs suspected to be infected with HPAIV. All samples were tested positive with H5 real-time RT-PCR with a mean CT value of 20.3 ± 5.46. Three samples were identified as H5N1 (CT value: 25.3 ± 8.5) and 10 samples as H5N8 (CT value: 20.1 ± 4.8) using type-specific real-time PCR. As the H5 RT-RPA assay was designed to amplify specific sequences of clade 2.2.1 H5 sequences (i.e. H5N1 viruses) but not clade 2.3.4.4b (i.e. H5N8 viruses) [11], only the three samples positive for H5N1 have developed amplification curves between 6 and 8 min with H5 RT-RPA assay. All H5N8 positive samples as well as the five negative controls either generated negative or invalid results. These results further confirm the specificity of the developed H5 RT-RPA assay on clinical samples. The inclusion of either type of EPC in the RT-RPA and real-time RT-PCR assays did not affect the specificity, sensitivity, and reproducibility of both assays.

CRediT authorship contribution statement

Basem M. Ahmed: Data curation, Investigation, Writing - original draft. **Haitham A. Amer:** Validation, Visualization, Writing - original draft. **Jonas Kissenkoetter:** Investigation, Software. **Ahmed Abd El Wahed:** Conceptualization, Supervision, Writing - review & editing. **Mahmoud M. Bayoumi:** Resources, Investigation. **Susane. Böhlken-Fascher:** Investigation. **Mahmoud A. Elgamal:** Investigation. **Nahed Yehia:** Resources, Investigation. **Ausama A. Yousif:** Validation, Supervision. **Mohamed A. Shalaby:** Conceptualization, Funding acquisition, Resources.

Declaration of competing interest

The author declares that he has no conflict of interest.

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References

[1] D.J. Alexander, I.H. Brown, History of highly pathogenic avian influenza, Rev. Sci. Tech. O I E 28 (2009) 19–38, https://doi.org/10.20506/rst.28.1.1856.
[2] G. Neumann, H5N1 influenza virulence, pathogenicity and transmissibility: what do we know? Future Virol. 10 (2015) 971–980, https://doi.org/10.2217/fvl.15.62.
[3] N. Yehia, M.M. Naguib, R. Li, N. Hagag, M. El-Husseiny, Z. Mosaad, A. Nour, N. Rabea, W.M. Hasan, M.K. Hassan, T. Harder, A.S.A. Arafa, Multiple introductions of reassorted highly pathogenic avian influenza viruses (H5N8) clade 2.3.4.4b causing outbreaks in wild birds and poultry in Egypt, Infect. Genet. Evol. 58 (2018) 56–65, https://doi.org/10.1016/j.meegid.2017.12.011.
[4] R.G. Webster, W.J. Bean, O.T. Gorman, T.M. Chambers, Evolution and ecology of influenza A viruses, Microbiol. Rev. 56 (1992) 152–179.
[5] D.L. Suarez, A. Das, E. Ellis, D.L. Suarez, A.A. Das, E.E. A, Review of rapid molecular diagnostic tools for avian influenza virus review of rapid molecular diagnostic tools for avian influenza virus, Avian Dis. 51 (2007) 201–208.
[6] A. Abd El Wahed, A. El-Deeb, M. El-Tholoth, H. Abd El Kader, A. Ahmed, S. Hassan, B. Hoffmann, B. Haas, M.A. Shalaby, F.T. Hufert, M. Weidmann, A portable reverse transcription recombinase polymerase amplification assay for rapid detection of foot-and-mouth disease virus, PLoS One 8 (2013) 1–7, https://doi.org/10.1371/journal.pone.0071642.
[7] O. Piepenburg, C.H. Williams, D.L. Stemple, N.A. Armes, DNA detection using recombination proteins, Plant Biol. 4 (2006) 2–8, https://doi.org/10.1371/journal.pbio.0040204.
[8] M. Euler, Y. Wang, D. Heidenreich, P. Patel, O. Strohmeier, S. Hakenberg, M. Niedrig, F.T. Hufert, M. Weidmann, Development of a panel of recombinase polymerase amplification assays for detection of biothreat agents, J. Clin. Microbiol. 51 (2013) 1110–1117, https://doi.org/10.1128/JCM.02704-12.
[9] M. Euler, Y. Wang, O. Nentwich, O. Piepenburg, F.T. Hufert, M. Weidmann, Recombinase polymerase amplification assay for rapid detection of Rift Valley fever virus, J. Clin. Virol. 54 (2012) 308–312, https://doi.org/10.1016/j.jcv.2012.05.006.
[10] H.M. Amer, A. Abd El Wahed, M.A. Shalaby, F.N. Almajhdi, F.T. Hufert, M. Weidmann, A new approach for diagnosis of bovine coronavirus using a reverse

transcription recombinase polymerase amplification assay, J. Virol. Methods 193 (2013) 337–340, https://doi.org/10.1016/j.jviromet.2013.06.027.
[11] N. Yehia, A.S. Arafa, A. Abd El Wahed, A.A. El-Sanousi, M. Weidmann, M.A. Shalaby, Development of reverse transcription recombinase polymerase amplification assay for avian influenza H5N1 HA gene detection, J. Virol. Methods 223 (2015) 45–49, https://doi.org/10.1016/j.jviromet.2015.07.011.
[12] M.A. Shalaby, A. El-deeb, M. El-tholoth, D. Hoffmann, C. Czerny, F.T. Hufert, M. Weidmann, A. Abd, E. Wahed, Recombinase polymerase amplification assay for rapid detection of lumpy skin disease virus, BMC Vet. Res. (2016) 10–15, https://doi.org/10.1186/s12917-016-0875-5.
[13] Z. Wang, P. Yang, Y.Z.K. Tian, C.B.J. Zhao, Development of a reverse transcription recombinase polymerase amplification combined with lateral - flow dipstick assay for avian influenza H9N2 HA gene detection, Transboundary Emerg. Dis. 66 (2018) 546–551, https://doi.org/10.1111/tbed.13063.
[14] M. Weidmann, M.P. Sanchez-seco, A.A. Sall, P. Ogo, Y. Thiongane, M.M. L, H. Schley, F.T. Hufert, Rapid detection of important human pathogenic Phleboviruses, J. Clin. Virol. 41 (2008) 138–142, https://doi.org/10.1016/j.jcv.2007.10.001.
[15] A.K. Witte, R. Sickha, P. Mester, S. Fister, D. Schoder, P. Rossmanith, Essential role of polymerases for assay performance – impact of polymerase replacement in a well-established assay, Biomol. Detect. Quantification 16 (2018) 12–20, https://doi.org/10.1016/j.bdq.2018.10.002.
[16] I.G. Wilson, Inhibition and facilitation of nucleic acid amplification, Appl. Environ. Microbiol. 63 (1997) 3741–3751.
[17] R. Sanders, S. Bustin, J. Huggett, D. Mason, Improving the standardization of mRNA measurement by RT-qPCR, Biomol. Detect. Quantification 15 (2018) 13–17, https://doi.org/10.1016/j.bdq.2018.03.001.
[18] M.F. Kavlick, Development of a universal internal positive control, Biotechniques 65 (2018) 275–280.
[19] J. Hoorfar, B. Malorny, A. Abdulmawjood, N. Cook, M. Wagner, P. Fach, Practical considerations in design of internal amplification controls for diagnostic PCR assays, J. Clin. Microbiol. 42 (2004) 1863–1868, https://doi.org/10.1128/JCM.42.5.1863.
[20] E. Ammar, C. Tsai, A.E. Whitfield, M.G. Redinbaugh, S.A. Hogenhout, Cellular and molecular aspects of rhabdovirus interactions with insect and plant hosts, Annu. Rev. Entomol. 54 (2009) 447–468, https://doi.org/10.1146/annurev.ento.54.110807.090454.
[21] C.F. Speller, B.M. Kemp, S.D. Wyatt, C. Monroe, W.D. Lipe, U.M. Arndt, D.Y. Yang, Ancient mitochondrial DNA analysis reveals complexity of indigenous North American Turkey domestication, Proc. Natl. Acad. Sci. U.S.A. 107 (2010) 2807–2812, https://doi.org/10.1073/pnas.0909724107.
[22] J. Li, J. Macdonald, F. Von Stetten, Review: a comprehensive summary of a decade development of the recombinase polymerase amplification, Analyst 144 (2019) 31–67, https://doi.org/10.1039/c8an01621f.
[23] B. Rohrman, R. Richards-kortum, Inhibition of recombinase polymerase amplification by background DNA: a lateral flow-based method for enriching target DNA, Anal. Chem. (2015), https://doi.org/10.1021/ac504365v.
[24] S. Kersting, V. Rausch, F.F. Bier, M. von Nickisch-Rosenegk, Multiplex isothermal solid-phase recombinase polymerase amplification for the specific and fast DNA-based detection of three bacterial pathogens, Microchimica Acta 181 (2014) 1715–1723, https://doi.org/10.1007/s00604-014-1198-5.
[25] Z.A. Crannell, A. Castellanos-gonzales, R. Mejia, A.C. White, R. Richards-kortum, Z. Crannell, A. Castellanos-gonzales, G. Nair, A multiplexed recombinase polymerase amplification assay to detect intestinal protozoa, Anal. Chem. (2015), https://doi.org/10.1021/acs.analchem.5b03267.
[26] H. Soliman, M. El-Matbouli, Rapid detection and differentiation of carp oedema virus and cyprinid herpes virus-3 in koi and common carp, J. Fish Dis. (2018) 1–12, https://doi.org/10.1111/jfd.12774.
[27] J. Chen, Y. Xu, H. Yan, Y. Zhu, L. Wang, Y. Zhang, Y. Lu, W. Xing, Sensitive and rapid detection of pathogenic bacteria from urine samples using multiplex recombinase polymerase amplification, Lab Chip 18 (2018) 2441–2452, https://doi.org/10.1039/c8lc00399h.
[28] H.Y. Lau, Y. Wang, E.J.H. Wee, J.R. Botella, M. Trau, Field demonstration of a multiplexed point-of- care diagnostic platform for plant pathogens, Anal. Chem. (2016), https://doi.org/10.1021/acs.analchem.6b01551.
[29] J.L. Muhammad, A. Hassan, M.R. Muhammad Sohail Sajid, A. Mahmood, Q. Abbas, G. Guan, H. Yin, J. Luo, Simultaneous detection of Theileria annulata and Theileria orientalis infections using recombinase polymerase amplification, Ticks Tick Borne Dis. (2018), https://doi.org/10.1016/j.ttbdis.2018.03.028.
[30] S. Kersting, V. Rausch, F.F. Bier, M. von Nickisch-Rosenegk, A recombinase polymerase amplification assay for the diagnosis of atypical pneumonia, Anal. Biochem. 550 (2018) 54–60, https://doi.org/10.1016/j.ab.2018.04.014.
[31] D.J. Bacich, K.M. Sobek, J.L. Cummings, A.A. Atwood, D.S. O'Keefe, False negative results from using common PCR reagents, BMC Res. Notes 4 (2011) 457, https://doi.org/10.1186/1756-0500-4-457.