Vietnam Journal of Food Control

A Scientific Journal of National Institute for Food Control
Journal homepage: http://vjfc.vn/

Research Article

Development and validation of a qPCR method for quantification of *Spodoptera litura* nucleopolyhedrovirus in plant protection products

Ninh Thi Hanh*, Nguyen Van Cuong, Nguyen Tuan Thanh, Tran Hung, Luong Thi Thuy
National Institute for Food Control, Hanoi, Vietnam

(Received: 27 Jan 2026; Revised: 12 Feb 2026; Accepted: 26 Feb 2026)

Abstract

Spodoptera litura nucleopolyhedrovirus (SpltNPV) is an insect virus belonging to the family Baculoviridae, with high specificity to the tobacco cutworm (*Spodoptera litura*), and is used in biological plant protection products. This study aimed to develop and validate a method for the detection and quantification of SpltNPV using real-time PCR. The method was developed based on amplification of the *lef-8* gene, which encodes a late expression factor that is highly conserved and specific to SpltNPV. The amplified target gene fragment was confirmed by Sanger sequencing to verify the reference strain, showing ≥ 99% similarity to the published *lef-8* sequence of SpltNPV. The real-time PCR method showed 100% accuracy, specificity, and sensitivity, with both the limit of detection and the limit of quantification reaching 10^2 OBs/g, and no amplification was observed in the negative control samples. The Ct-$\log_{10}$ viral DNA concentration standard curve demonstrated good linear correlation (R^2 = 0.995). The repeatability and reproducibility parameters met the acceptance criteria according to CEN/TS 17329-1:2021 and ISO 22118:2011. The results indicate that the proposed method has high reliability and stability and is suitable for the detection, quantification, and quality control of SpltNPV in biological plant protection products.

Keywords: *Spodoptera litura nucleopolyhedrovirus; real-time PCR; SpltNP; plant protection product.*

1. INTRODUCTION

The tobacco cutworm (*Spodoptera litura* Fabricius, Lepidoptera: Noctuidae) is a serious polyphagous pest widely distributed in the Asia-Pacific region, causing damage to more than 150 different plant species, particularly vegetables, leguminous crops, and short-term industrial crops. This insect species has high reproductive capacity, a short life cycle, and strong feeding activity during the larval stage, frequently causing pest outbreaks and leading to severe losses in agricultural productivity [1, 2]. The prolonged and excessive use of chemical insecticides for the control of *S. litura* has rapidly increased resistance, while also causing numerous negative impacts on the environment and human health [3, 4].

In this context, biological plant protection products based on baculoviruses are considered a safe and sustainable alternative. *S. litura* nucleopolyhedrovirus (SpltNPV), belonging to the family Baculoviridae, is a double-stranded DNA virus with high host specificity, primarily infecting *S. litura* and causing little to no effect on non-target organisms [5, 6]. The virus infects larvae through ingestion of viral occlusion bodies (OBs). In the midgut, the OBs dissolve, releasing virions, initiating infection and systemic replication, ultimately leading to host death [6]. The control efficacy of SpltNPV-based products depends directly on the OB content in the product and the OB dose applied per unit area under field conditions [7].

Currently, quantification of SpltNPV in biological products is still mainly based on microscopic counting of OBs using a counting chamber, which has limitations in throughput and accuracy and is highly dependent

*Corresponding author: Ninh Thi Hanh (E-mail: ninhhanh891997@gmail.com)
https://doi.org/10.47866/2615-9252/vjfc.4671
Copyright © 2025 The author(s). This article is licensed under CC BY-NC 4.0

on the operator [8]. Quantitative real-time PCR, with its advantages in sensitivity, specificity, and accurate quantification of viral DNA, is considered a suitable tool to replace traditional methods [9, 10]. However, fully standardized and validated real-time PCR methods for quantifying SpltNPV in plant protection products remain limited, particularly in Vietnam. Therefore, the development and optimization of a suitable analytical method are necessary to support quality control and effective management of SpltNPV-based biological products [11].

2. MATERIALS AND METHODS

2.1. Research object/Materials

The study was conducted using a quality control (QC) sample consisting of powdered SpltNPV material manufactured by Henan Jiyuan Baiyun Industry Co., Ltd. The QC sample was imported from China and stored frozen during transport to ensure the integrity of the virus and the content of viral OBs. According to the technical information provided by the manufacturer, the nominal concentration of the QC sample was 1.3×10^{11} OBs/g (Certificate of Analysis - SpltNPV, Batch No.: 241112).

2.2. Chemicals and equipment

The chemicals and reagents used in this study included the QIAamp MinElute Virus Kit (Qiagen, USA), Luminaris HiGreen qPCR Master Mix (2X) (Thermo Fisher Scientific, USA), primers (IDT, USA), real-time PCR tube strips (Thermo Fisher Scientific, USA), ExoSAP-IT™ PCR Product Cleanup Reagent (Thermo Fisher Scientific, USA), and the BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, USA). All other chemicals met the requirements for molecular biology research.

The main instruments and equipment used in the study included the CFX96 Touch Real-Time PCR Detection System (Bio-Rad, USA), Hettich MIKRO 220R refrigerated centrifuge (Hettich, Germany), NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, USA), Neubauer Improved counting chamber (Marienfeld, Germany), SeqStudio Genetic Analyzer 3500 sequencing system (Applied Biosystems, USA), vortex mixer (IKA, Germany), spin-down centrifuge (GeneReach, Taiwan), and other laboratory equipment.

2.3. Research methods

2.3.1. Viral OBs purification and quantification using a counting chamber

The QC sample was purified by centrifugation to remove the supernatant, followed by resuspension of the pellet in 0.1% SDS. The suspension was then centrifuged again to collect the pellet containing viral OBs [12]. The purified OB suspension was serially diluted tenfold and quantified using a counting chamber by counting the number of OBs in five large squares, each consisting of 16 small squares. The counting results were used to calculate the OB concentration and to construct the standard curve for real-time PCR. The number of OBs was calculated using the following formula:

$$Total\ number\ of\ OB\ per\ mL = \frac{Mean\ of\ OBs\ in\ a\ large\ square\ \times Dilution\ factor\ \times 10^3}{Area\ of\ a\ large\ square\ \times\ Depth\ of\ chamber}$$

2.3.2. DNA extraction and target gene sequencing - Sanger sequencing for virus identification

The DNA of SpltNPV was extracted according to the protocol of the QIAamp MinElute Virus Kit (Qiagen, USA). The concentration of total SpltNPV DNA was quantified using a NanoDrop 1000 spectrophotometer at a wavelength of 260 nm, with an OD260/OD280 ratio ranging from 1.8 to 2.0. The DNA solution was stored at -20℃ until use.

Sequencing of the target gene fragment was performed according to the BigDye Terminator v3.1 Cycle Sequencing Kit Protocol (PN 4337035) (Thermo Fisher Scientific, USA) to confirm the positive control strain. The target gene fragment was sequenced by amplifying a 217 bp fragment of the late expression factor 8 (*lef-8*) gene of SpltNPV (GenBank Accession No. AY706581) using the SlNPVlef-8 primer pair [13]. The DNA sequence corresponding to the target gene was compared for sequence similarity against the U.S. GenBank database using the BLAST tool.

2.3.3. Real-time PCR method for amplification of the target gene

The method was designed based on the amplification of a 217 bp fragment of the late expression factor 8 (*lef-8*) gene of SpltNPV (GenBank Accession No. AY706581) using the primer pair SlNPVlef-8Fnes: 5′-ACGAAAGCATCTAGTGCG-3′ and SlNPVlef-8Rnes: 5′-CCGAGCTTTGATTTGAGC-3′ [13]. SpltNPV is

a double-stranded DNA virus, and the *lef-8* gene is present as a single copy per genome; therefore, the number of *lef-8* copies directly reflects the amount of viral genome in the sample [14]. Viral occlusion bodies package multiple virions; thus, real-time PCR results are correlated with OB content when the sample processing procedure is standardized. Due to variation in the number of virions per OB, the method was developed and validated using an OB-based standard curve for the quantification and quality control of powdered products containing SpltNPV.

The real-time PCR reaction was performed in a total volume of 20 µL, consisting of 10 µL of Luminaris HiGreen qPCR Master Mix (2X), 2 µL of forward primer (10 µM), 2 µL of reverse primer (10 µM), 1 µL of DNA template (100 - 200 ng), and nuclease-free water. The reaction conditions consisted of an initial denaturation step at 95°C for 10 min, followed by 45 cycles of denaturation at 95°C for 30 s and annealing at 62°C for 1 min, with the melting curve set at 95°C for 15 s.

2.3.4. Method validation

The method validation parameters were established in accordance with CEN/TS 17329-1:2021, which specifies the validation of real-time PCR methods, and ISO 22118:2011, which guides performance criteria and method validation for the detection, identification, and quantification of specific DNA sequences in food. The validation parameters included the 95% limit of detection (LOD_{95}), accuracy (AC ≥ 90%), specificity (SP ≥ 90%), sensitivity (SE ≥ 90%), limit of quantification (LOQ), repeatability (≤ 25%), and reproducibility (≤ 35%).

3. RESULTS AND DISCUSSION

3.1. Quantification of SpltNPV using a counting chamber

The OBs of SpltNPV, after being purified from the plant protection product sample by multi-step centrifugation combined with dilution in 0.1% SDS solution, were quantified using a Neubauer Improved counting chamber (Marienfeld, Germany). The sample was diluted 10^{-4} for counting. Counting was performed in five large squares, each consisting of 16 small squares, with each small square having an area of 0.0025 mm² and a counting chamber depth of 0.1 mm. The experiment was independently repeated three times to ensure the reliability of the results. The detailed results are presented in **Table 1**.

Table 1. *Observed OB counts and converted the density of SpltNPV*

Trial	Number of viral occlusion bodies counted in five large squares					Mean OB count	OB concentration (OBs/g)
	1	2	3	4	5		
1	54	49	56	48	51	51.6	1.3×10^{11}
2	51	53	51	54	53	52.4	1.3×10^{11}
3	54	50	57	53	56	54.0	1.4×10^{11}

The quantification of SpltNPV using the Neubauer counting chamber showed that the number of OBs in the five large squares varied slightly among experimental replicates, with mean values of 51.6, 52.4, and 54.0 OBs, respectively. The minor differences among counting positions and between replicates indicate that the purified viral occlusion body suspension was highly homogeneous and that the selected 10^{-4} dilution was appropriate, ensuring that the number of OBs fell within the optimal counting range of the Neubauer chamber.

Based on the mean counts, the concentration of viral occlusion bodies in the QC sample was converted to approximately 1.3×10^{11} OBs/g in all three experimental replicates. This result was consistent with the nominal concentration declared by the manufacturer, thereby confirming that the QC sample met the required quality specifications and demonstrating the repeatability and reliability of the counting method. On this basis, the obtained data were used as reference values to prepare the standard dilution series for the real-time PCR assay.

3.2. Confirmation of *Spodoptera litura* nucleopolyhedrovirus

To definitively confirm that the sample contained the SpltNPV, the *lef-8* gene fragment was amplified from viral DNA and purified for the Sanger sequencing method. The sequencing results and comparison with the reference sequence available in NCBI are shown in **Figure 1**.

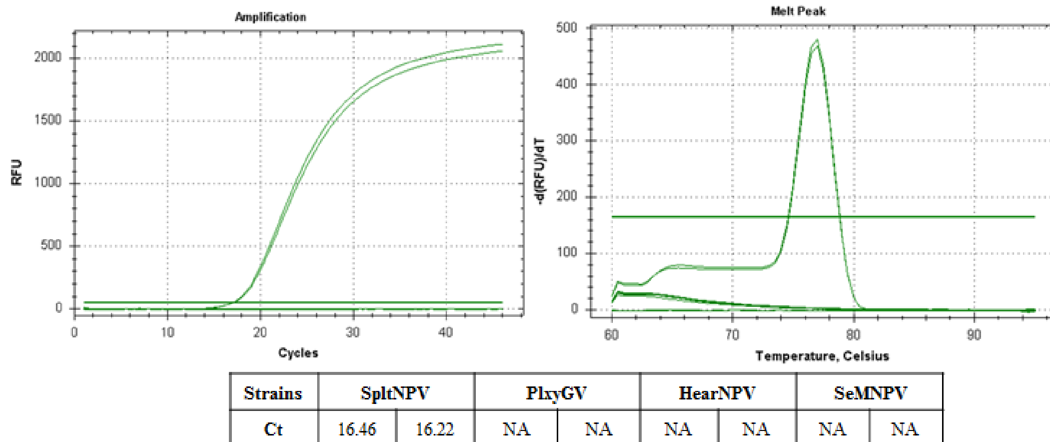
```
Spodoptera litura NPV isolate A17-3 lef-8 gene, partial cds
Sequence ID: AY706580.1   Length: 677   Number of Matches: 1
   See 1 more title(s)   See all Identical Proteins(IPG)

Range 1: 215 to 395  GenBank   Graphics                         Next Match   Previous Match

Score              Expect    Identities        Gaps          Strand
329 bits(178)      8e-86     180/181(99%)      0/181(0%)     Plus/Minus

Query  1    CAACAATAGATTAGCCGTTGTGGTACTCGTACGGAGTGAGCAGAGCCGAAATGTTAACTT  60
            |||||||||||| |||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  395  CAACAATAGATTCGCCGTTGTGGTACTCGTACGGAGTGAGCAGAGCCGAAATGTTAACTT  336

Query  61   TGTCGTTTATTAGAATATGTTTGCGTATACATATCATGCCTTCGTGATGATTGACAAATA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  335  TGTCGTTTATTAGAATATGTTTGCGTATACATATCATGCCTTCGTGATGATTGACAAATA  276

Query  121  GAATATTTTTAAACAGTTTGATCTCGACGGGAAACCGTTTGCGTTTAAACTCGTACACGA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  275  GAATATTTTTAAACAGTTTGATCTCGACGGGAAACCGTTTGCGTTTAAACTCGTACACGA  216

Query  181  T  181
            |
Sbjct  215  T  215
```

Figure 1. *Comparison between the obtained genomic sequence and the reference sequence in NCBI*

The sequence of the target *lef-8* gene fragment (217 bp), amplified using the SlNPVlef8 primer pair, was sequenced and analyzed by BLASTN. The results showed 100% query coverage and very high sequence similarity (99%; 180/181 bp) to nucleopolyhedrovirus sequences belonging to the *Spodoptera* group in GenBank, including the SpltNPV reference sequence (GenBank Accession No. AY706580.1). These findings, together with the fact that *lef-8* is a conserved core gene of baculoviruses, confirm that the virus present in the QC sample was SpltNPV. This result provides a reliable basis for using the total DNA material obtained from the QC sample in subsequent studies, particularly for the development and validation of a real-time PCR method for assessing SpltNPV content in product quality control.

3.3. Real-time PCR - Evaluation of the amplification capability of the SlNPVlef-8 primer pair

The purified total DNA was used as the template for the real-time PCR reaction with the primer pair specific to the *lef-8* gene, in order to evaluate the amplification capability of the SlNPVlef-8 primer pair and the suitability of the thermal cycling conditions for the detection of SpltNPV. The results are presented in **Figure 2**.



Strains	SpltNPV		PlxyGV		HearNPV		SeMNPV	
Ct	16.46	16.22	NA	NA	NA	NA	NA	NA

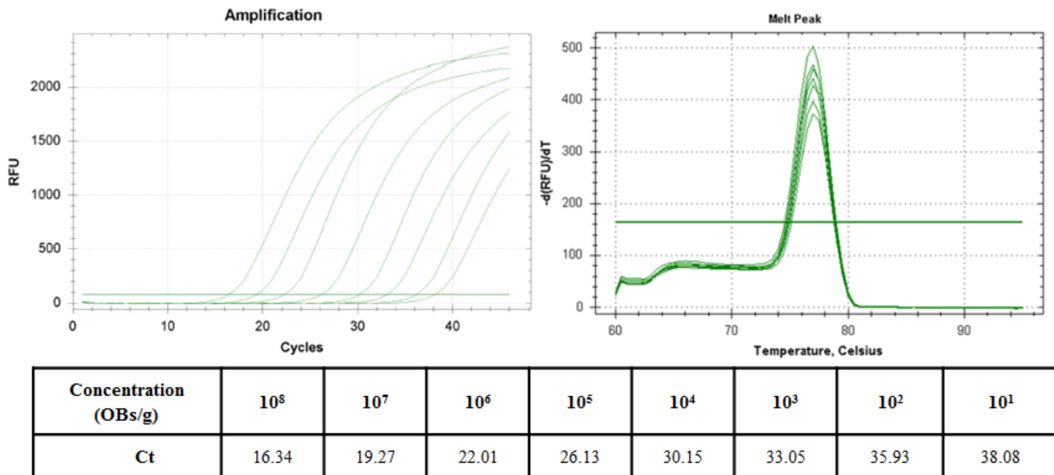
Figure 2*. Amplification curve (left) and corresponding melting curve (right) of primer-testing sample*

The real-time PCR analysis showed that the SlNPVlef-8 primer pair efficiently amplified the positive control sample (10^8 OBs/g), as demonstrated by sigmoidal amplification curves with corresponding Ct values of 16.46 and 16.22 (**Figure 2**), which were consistent with the high DNA template concentration. Melting curve analysis revealed a single melting temperature (Tm) peak at 77.0°C, indicating that the amplification product was highly specific and that no non-specific products or primer dimers were observed. These results confirm the feasibility of the real-time PCR assay using the SlNPVlef-8 primer pair, providing a basis for further optimization of the reaction conditions and construction of the standard curve for method validation.

3.4. Method Validation

3.4.1. Limit of Detection of the Method (LOD)

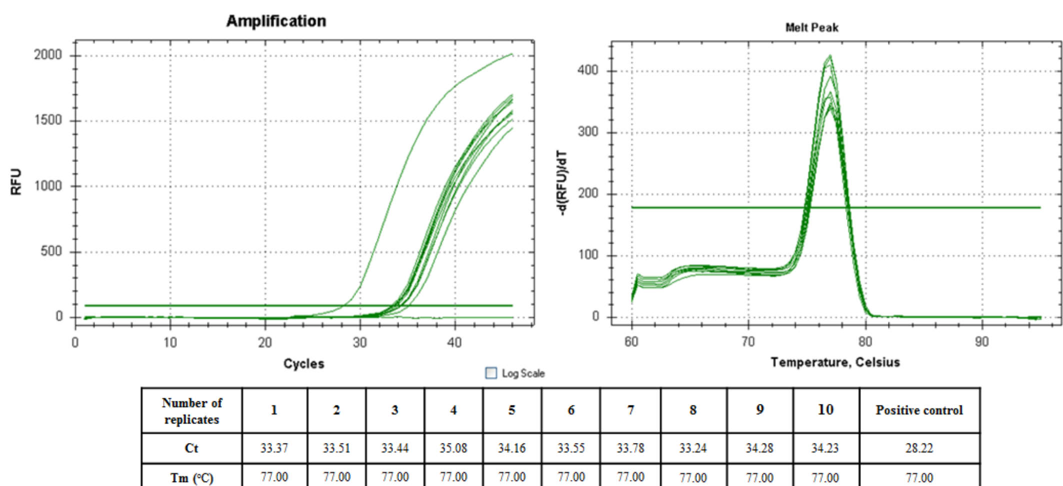
To determine the LOD, total DNA extracted from 1 g of samples containing different SpltNPV concentrations, including 10^8, 10^7, 10^6, 10^5, 10^4, 10^3, 10^2, and 10^1 OBs/g, was used as the template in the real-time PCR reaction with the specific primer pair for amplification of the *lef-8* gene. The obtained results are shown in **Figure 3**.



Concentration (OBs/g)	10^8	10^7	10^6	10^5	10^4	10^3	10^2	10^1
Ct	16.34	19.27	22.01	26.13	30.15	33.05	35.93	38.08

Figure 3. *Results of the limit of detection assessment of the method at different OB concentrations (10^1 - 10^8 OBs/g)*

Figure 3 indicates that, at the viral concentration of 10^2 OBs/g, the cycle threshold (Ct) was approximately 35. Therefore, the viral concentration of 10^2 OBs/g was predicted to be the lowest concentration detectable by the method. To determine the limit of detection, the reaction was repeated 10 times at the viral concentration of 10^2 OBs/g. The results presented in **Figure 4** showed that the positive detection rate at 10^2 OBs/g was 100%. This result is fully consistent with previous studies on *Helicoverpa armigera* nucleopolyhedrovirus (HearNPV) and *Spodoptera exigua* nucleopolyhedrovirus (SeNPV), which demonstrated that real-time PCR systems targeting conserved genes involved in transcription/replication, including *lef-8*, *lef-9*, or DNA polymerase, generally exhibit high sensitivity, with detection limits in the range of 10^1 - 10^3 OBs/g or OBs/mL, depending on the sample matrix and DNA extraction procedure [15, 16]. Therefore, the LOD was determined to be 10^2 OBs/g.

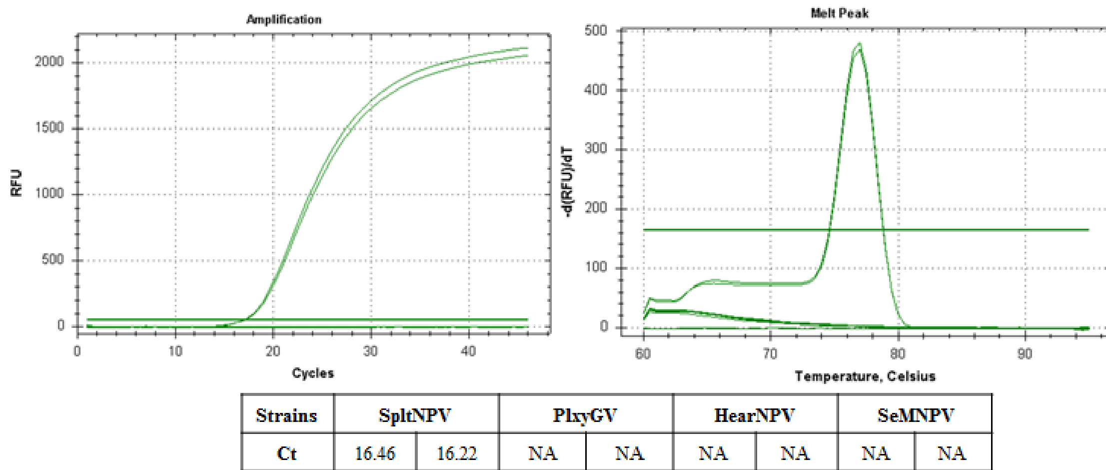


Number of replicates	1	2	3	4	5	6	7	8	9	10	Positive control
Ct	33.37	33.51	33.44	35.08	34.16	33.55	33.78	33.24	34.28	34.23	28.22
Tm (°C)	77.00	77.00	77.00	77.00	77.00	77.00	77.00	77.00	77.00	77.00	77.00

Figure 4. *Amplification curves (left) and corresponding melting curves (right) from 10 replicates of the sample containing 10^2 OBs/g*

3.4.2. Accuracy (AC), specificity (SP), and sensitivity (SE)

The amplification performance of the real-time PCR method was evaluated to determine its accuracy, specificity, and sensitivity by comparing the reaction against the target virus, SpltNPV, and non-target viruses, including *Plutella xylostella* granulovirus (PlxyGV), HearNPV, and SeMNPV. These non-target viruses were selected based on their biological and taxonomic relatedness to SpltNPV, including: a different lineage within the same family for PlxyGV, the same lineage but a different host for HearNPV, and the same genus *Spodoptera* with close genetic relatedness for SeMNPV. These viruses are all commonly used in biological plant protection products; therefore, they are suitable for evaluating the accuracy, specificity, and sensitivity of the method.



Strains	SpltNPV		PlxyGV		HearNPV		SeMNPV	
Ct	16.46	16.22	NA	NA	NA	NA	NA	NA

***Figure 5**. Amplification curves (left) and corresponding melting curves (right) of the viral species*

Figure 5 indicates that the SpltNPV sample exhibited sigmoidal fluorescent amplification curves, with corresponding Ct values of 16.22 and 16.46, whereas no amplification signal was detected in the non-target virus samples (Ct = NA) under the same reaction conditions. Melting curve analysis showed a single Tm peak corresponding to the amplification product of SpltNPV (Tm = 77.0°C), while no characteristic peak was observed in the non-target samples. These results demonstrate the high specificity of the reaction and confirm the selective detection capability of the developed real-time PCR method for SpltNPV, thereby satisfying the requirements for evaluating accuracy, sensitivity, and specificity in subsequent validation steps.

3.4.3. Construction of the standard curve

The quantitative real-time PCR method was established based on the linear relationship between the target DNA concentration in the sample and the threshold cycle (Ct) value. The standard curve was generated using the analysis software of the CFX96 Touch Real-Time PCR Detection System (Bio-Rad, USA) in **Figure 6**.

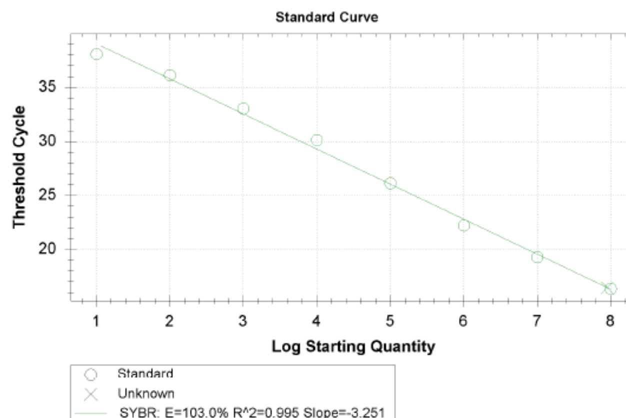
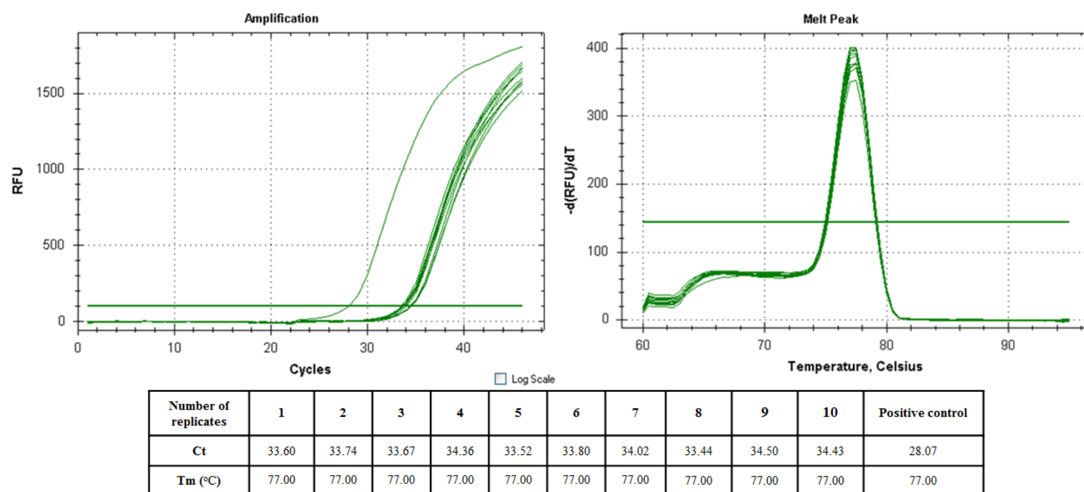


***Figure 6**. Standard curve at concentrations ranging from 10^1 to 10^8 OBs/g*

The results showed a strong linear correlation between the threshold cycle and the $\log_{10}$ of the target DNA concentration in the sample, with a coefficient of determination (R^2) of 0.995. The slope of the standard curve was -3.251, and the amplification efficiency was 103.0%; these parameters were all within the acceptable range for real-time PCR analysis [17]. The linear regression equation of the standard curve was Y = -3.251X + 41.832, where Y represents the Ct value, and X represents the $\log_{10}$ of the initial target DNA copy number (Sq - starting quantity). Based on this equation, the initial number of OBs in the sample was calculated using the following formula: Sq = 10 [(Ct - 41.832)/-3.251].

3.4.4. Limit of quantification (LOQ)

Based on the lowest concentration detectable by the method, the limit of quantification (LOQ) was determined by repeating the experiment 10 times using samples with an OB density of 10^2 OBs/g. The results presented in **Figure 7** showed that all 10 replicates were positive, corresponding to a 100% positive detection rate. Compared with previous studies, the level of 10^2 OBs/g obtained in this study falls within the commonly reported sensitivity range of real-time PCR systems used for baculovirus quantification [15, 16]. Therefore, it can be concluded that the LOQ is 10^2 OBs/g.

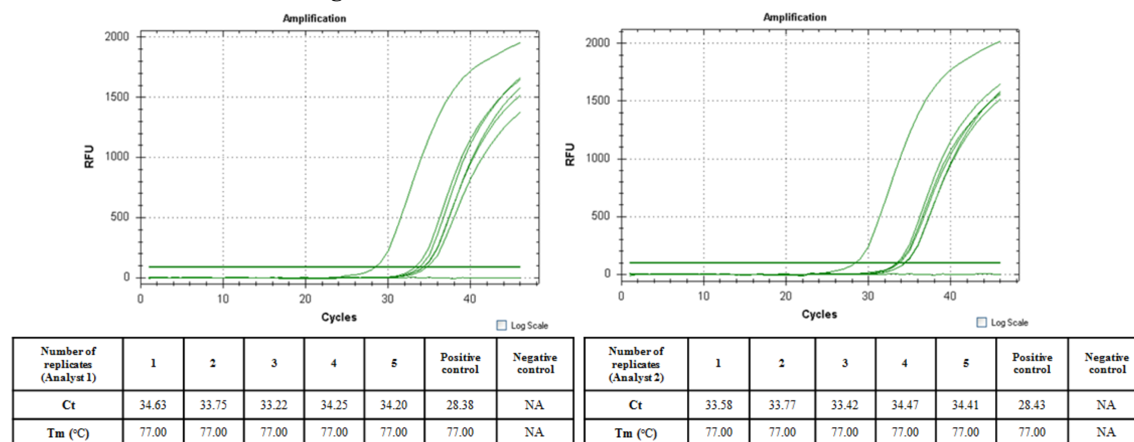


Number of replicates	1	2	3	4	5	6	7	8	9	10	Positive control
Ct	33.60	33.74	33.67	34.36	33.52	33.80	34.02	33.44	34.50	34.43	28.07
Tm (°C)	77.00	77.00	77.00	77.00	77.00	77.00	77.00	77.00	77.00	77.00	77.00

***Figure** 7. Amplification curves (left) and corresponding melting curves (right) from 10 replicates of the sample containing 10^2 OBs/g.*

3.4.5. Repeatability (RSDr) and reproducibility (RSDR)

To determine the repeatability (RSDr) and reproducibility (RSDR) of the method, experiments were performed in five replicates per sample, and reproducibility conditions were assessed using two analysts. The obtained results are shown in **Figure 8**.



Number of replicates (Analyst 1)	1	2	3	4	5	Positive control	Negative control
Ct	34.63	33.75	33.22	34.25	34.20	28.38	NA
Tm (°C)	77.00	77.00	77.00	77.00	77.00	77.00	NA

Number of replicates (Analyst 2)	1	2	3	4	5	Positive control	Negative control
Ct	33.58	33.77	33.42	34.47	34.41	28.43	NA
Tm (°C)	77.00	77.00	77.00	77.00	77.00	77.00	NA

***Figure** 8. Amplification curves of five replicates at a concentration of 10^2 OBs/g performed by two analysts*

Figure 8 showed relatively consistent amplification curves among replicates for both analysts, reflecting the stability of the system under the investigated conditions. The Ct values obtained by Analyst 1 ranged from 33.22 to 34.63, with a mean of approximately 34.01 and a standard deviation (SD) of approximately 0.54, while those obtained by Analyst 2 ranged from 33.42 to 34.47, with a mean of approximately 33.93 and an SD of approximately 0.48. The mean Ct difference between the two analysts was only approximately 0.08 cycles, indicating a low level of inter-analyst variation.

The repeatability values for Analysts 1 and 2 were 0.0044 and 0.0039, respectively, both of which were lower than the acceptance threshold of 0.25. This indicates low dispersion and good stability of the assay under repeatability conditions. In addition, the reproducibility between the two analysts was 0.0032, satisfying the requirement of being less than 0.35. These results indicate that the precision between analysts met the acceptance criterion and that inter-laboratory/operator variation was negligible. Furthermore, melting curve analysis showed a consistent Tm of 77.0°C across all replicates, indicating that the amplification product was specific and stable. The positive control has Ct values of approximately 28.38 to 28.43 with corresponding Tm values, whereas the negative control showed no signal (NA), thereby confirming the reliability of the reaction conditions and contamination control.

4. CONCLUSION

The quantification results obtained using the Neubauer counting chamber and the identification results for SpltNPV demonstrated that the QC sample was reliable for use as a reference material during the development of the detection and quantification method. On this basis, a real-time PCR method targeting the *lef-8* gene was developed and evaluated. The method showed 100% accuracy, specificity, and sensitivity, with the LOD and LOQ of 10^2 OBs/g. The real-time PCR standard curve demonstrated a strong linear correlation between Ct values and the $\log_{10}$ virus concentration ($R^2 = 0.995$), with an amplification efficiency of 103.0%. In addition, the repeatability values (0.0044 and 0.0039) and reproducibility value (0.0032) were all within the acceptable limits according to CEN/TS 17329-1:2021 and ISO 22118:2011, demonstrating good precision and accuracy of the method.

Overall, the results indicate that the proposed real-time PCR method is highly reliable and practically applicable for quality control and quantification of SpltNPV in powdered biological plant protection products. This contributes to the standardization of procedures for assessing viral content and improving the management and use of virus-based products in sustainable agricultural production.

ACKNOWLEDGEMENTS

This research was funded by the National Institute for Food Control, project code NIFC.ĐTCS.24.10. We sincerely thank the Department of Food microbiology and Genetically modified food, the Department of Experimental on Animals and Plants, and their staff for their support in conducting this research.

REFERENCES

[1] K. Maes, *"Spodoptera litura* (taro caterpillar)" *CABI Compendium*, CAB International, Wallingford, UK, 2014.

[2] D.S. Hill, "Agricultural insect pests of the tropics and their control - Second edition," *Cambridge University Press*, Cambridge, 1987.

[3] M. Ahmad, M. I. Arif, and M. Ahmad, "Occurrence of insecticide resistance in field populations of *Spodoptera litura* (Lepidoptera: Noctuidae) in Pakistan," *Crop Protection*, vol. 26, pp. 809-817, 2007.

[4] World Health Organization, "Public health impact of pesticides used in agriculture," *WHO Press*, Geneva, 1990.

[5] G. W. Blissard and G. F. Rohrmann, "Baculovirus diversity and molecular biology," *Annual Review of Entomology*, vol. 35, pp. 127-155, 1990.

[6] G. F. Rohrmann, *Baculovirus Molecular Biology, 4th ed*. Bethesda, MD, USA: National Center for Biotechnology Information, 2019.

[7] M. Gani, R. K. Gupta, and K. Bali, "Efficacy of Spodoptera litura multiple nucleopolyhedrovirus after serial passage through the homologous insect larval host," *Indian Journal of Experimental Biology*, vol. 52, pp. 369-374, 2014.

[8] Rohrmann GF. Baculovirus Molecular Biology [Internet]. 4th edition. Bethesda (MD): National Center for Biotechnology Information (US); 2019. Chapter 2, Structural proteins of baculovirus occlusion bodies and virions. Available from: https://www.ncbi.nlm.nih.gov/sites/books/NBK543455/. Rogers-Broadway

[9] C. S. Molina-Ruiz, J. A. Zamora-Briseño, O. Simón, R. Lasa, and T. Williams, "A qPCR assay for the quantification of selected genotypic variants of *Spodoptera frugiperda* multiple nucleopolyhedrovirus (Baculoviridae)," *Viruses*, vol. 16, no. 6, 2024.

[10] M. P. Zwart, M. M. van Oers, J. S. Cory, J. W. M. van Lent, W. van der Werf, and J. M. Vlak, "Development of a quantitative real-time PCR for determination of genotype frequencies for studies in baculovirus population biology," *Journal of Virological Methods*, vol. 148, no. 1-2, pp. 146-154. 2008.

[11] T. H. H. Le et al., "Occlusion bodies purity of nuclear polyhedrosis virus (NPV) and product," *Journal of Science of the Open University of Ho Chi Minh City - Engineering and Technology*, vol. 14, no. 1, pp. 16-26, 2019 (in Vietnamese).

[12] I. Nazli-Huda, A. Sajap, and W. Lau, "Stability of *Spodoptera litura* nucleopolyhedrovirus in sodium dodecyl sulphate," *Afr. J. Biotechnol*., vol. 11, no. 16, pp. 3877-3881, 2012.

[13] Kouassi, L.N., Tsuda, K., Goto, C., Mukawa, S., Sakamaki, Y., Kusigemati, K., Nakamura, M., "Prevalence of latent virus in *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) and its activation by a heterologous virus," *Applied Entomology and Zoology* 2009, *44*, 95-102.

[14] J. Jose, S. K. Jalali, T. M. Shivalingaswamy, N. K. Krishna Kumar, R. Bhatnagar, and A. Bandyopadhyay, "Molecular characterization of nucleopolyhedrovirus of three Lepidopteran pests using late expression factor-8 gene," *Indian Journal of Virology*, vol. 24, no. 1, pp. 59-65, 2013.

[15] J. A. Jehle, G. W. Blissard, B. C. Bonning, J. S. Cory, E. A. Herniou, G. F. Rohrmann, D. A. Theilmann, S. M. Thiem, and J. M. Vlak, "On the classification and nomenclature of baculoviruses: A proposal for revision," *Archives of Virology*, vol. 151, no. 7, pp. 1257-1266, 2006.

[16] R. B. Hitchman, E. A. Siaterli, C. P. Nixon, and L. A. King, "Quantitative real-time PCR for rapid and accurate titration of recombinant baculovirus particles," *Biotechnology and Bioengineering*, vol. 96, no. 4, pp. 810-819, 2007.

[17] K. R. Rogers-Broadway and E. Karteris, "Amplification efficiency and thermal stability of qPCR instrumentation: Current landscape and future perspectives," *Experimental and Therapeutic Medicine*, vol. 10, no. 4, pp. 1261, 2015.