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Research Article

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

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Abstract

Spodoptera exigua multiple nucleopolyhedrovirus (SeMNPV) is an important biological control agent widely used as an eco-friendly alternative to chemical pesticides for managing *Spodoptera exigua*. The efficacy of SeMNPV-based plant protection products strongly depends on the concentration of viral occlusion bodies (OBs); however, conventional quantification methods based on hemocytometer counting are labor-intensive, time-consuming, and subject to operator bias. Therefore, this study aimed to develop and validate a real-time PCR (qPCR) method for the quantification of SeMNPV in plant protection products. In this study, a qPCR-based method for the quantification of SeMNPV was developed by targeting amplification of the *dnapol* gene, which represents a highly conserved and species-specific genetic marker for this virus. Viral identity was confirmed by *dnapol* gene sequencing, and hemocytometer-based quantification of viral OBs was employed to determine reference input values for qPCR validation. Method validation was conducted in accordance with ISO 22118:2011 guidelines, evaluating analytical sensitivity, specificity, accuracy, linearity, repeatability, and reproducibility. The assay demonstrated high sensitivity, with both the limit of detection and the limit of quantification established at 10^2 OBs/mL. Specificity, accuracy, and sensitivity reached 100%, with no cross-reactivity observed against non-target baculoviruses. A robust standard curve based on the linear relationship between Ct values and OB concentration showed excellent linearity (R^2 = 0.994), a slope of -3.151, and an amplification efficiency of 107.6%. Repeatability and reproducibility met acceptance criteria, indicating good precision and method stability. Overall, this study presents the first validated qPCR method for SeMNPV quantification, validated against occlusion body counts, providing a sensitive, specific, and biologically relevant tool for quality control and regulatory assessment of SeMNPV-based biopesticides.

Keywords: *Spodoptera exigua multiple nucleopolyhedrovirus, SeMNPV, qPCR, plant protection product.*

1. INTRODUCTION

The beet armyworm (*Spodoptera exigua*) is a highly destructive agricultural pest with a wide host range, causing significant yield losses in vegetable and field crops worldwide [1, 2]. In many regions, including Southeast Asia, intensive use of chemical pesticides has led to serious concerns regarding environmental contamination, human health risks, and the rapid development of pesticide resistance in *S. exigua* populations [3, 4]. These challenges have driven increasing interest in biological plant protection products as sustainable and environmentally friendly alternatives.

Among biological control agents, *S. exigua* multiple nucleopolyhedrovirus (SeMNPV), a member of the family Baculoviridae, has been widely recognized for its high host specificity, safety to non-target organisms, and effectiveness against larval stages of *S. exigua* [5]. SeMNPV-based biopesticides exert their insecticidal activity through ingestion of viral occlusion bodies (OBs), which initiate infection in the insect midgut and

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lead to systemic viral replication and host death [6]. The insecticidal efficacy of these products is therefore strongly dependent on the concentration of OBs present in the formulation. Accurate quantification of SeMNPV is essential for ensuring product quality, consistency, and field performance. Conventional quantification methods, such as hemocytometer-based counting of OBs, are commonly used but suffer from several limitations, including low throughput, high labor demand, and significant operator-dependent variability [7]. These constraints limit their suitability for routine quality control and regulatory monitoring of SeMNPV-based plant protection products.

Quantitative real-time polymerase chain reaction (qPCR) offers a powerful alternative for virus quantification, providing high sensitivity, specificity, and rapid turnaround time. By targeting conserved viral genes, qPCR enables precise measurement of viral DNA across diverse sample matrices [8]. Among the genes involved in baculovirus replication, the DNA polymerase (*dnapol*) gene is the most highly conserved across the family Baculoviridae and has been identified as an ancestral core gene based on phylogenetic analyses, making it an ideal molecular target for accurate and reliable quantification of SeMNPV [9]. Despite its widespread application in virology, standardized and validated qPCR methods for quantifying SeMNPV in commercial plant protection products remain limited, particularly in Vietnam. Therefore, the present study aimed to develop and validate a qPCR-based method for the quantification of SeMNPV in plant protection products. The method was designed to support reliable quality control, contribute to standardization efforts, and facilitate the broader adoption of SeMNPV-based biopesticides in sustainable agricultural systems.

2. MATERIALS AND METHODS

2.1. Reference material and reagents

2.1.1. Quality control material

The study was conducted using a quality control (QC) sample consisting of liquid *S. exigua* nucleopolyhedrovirus material produced by Henan Jiyuan Baiyun Industry Co., Ltd. The QC sample was imported from China and maintained under frozen conditions during transportation to preserve viral integrity and the concentration of viral OB. According to the technical information provided by the manufacturer, the nominal content of the QC sample was 2.0×10^{10} SeNPV OBs/mL (Certificate of Analysis - SeNPV, Batch No.: 241003).

2.1.2. Reagents

Viral DNA was extracted using the QIAamp MinElute Virus Kit (Qiagen, USA). The qPCR reaction mixture consisted of Luminaris HiGreen qPCR Master Mix (2X) (Thermo Fisher Scientific, USA), sequence-specific primers synthesized by Phusa (Phusa, Vietnam), nuclease-free water, and optical qPCR tube strips (Thermo Fisher Scientific, USA). ExoSAP-IT PCR Product Cleanup Reagent (Thermo Fisher Scientific, USA) was used for gene sequencing.

2.2. Equipments

All qPCR assays were performed on the CFX96 Touch Real-Time PCR Detection System (Bio-Rad, USA). Additional laboratory equipment included the Hettich MIKRO 220R refrigerated centrifuge (Hettich, Germany), the NanoDrop spectrophotometer (Thermo Fisher Scientific, USA) for DNA quantification, the vortex mixer (IKA, Germany), the mini centrifuge (GeneReach, Taiwan), SeqStudio 3500 (Thermo Fisher Scientific, USA0), Neubauer chamber (Marienfeld, Germany) and other standard ancillary laboratory instruments.

2.3. Research methods

2.3.1. Purification and hemocytometer-based quantification of SeMNPV

Reference material was centrifuged at 1,500 rpm for 5 min to remove the supernatant. The pellet was resuspended in 100 mL of sterile distilled water or 0.1% SDS solution and centrifuged again under the same conditions. The supernatant was discarded, and the purified, opaque-white viral occlusion bodies (OBs) pellet was retained, following the method of Nazli-Huda [10].

Purified viral suspensions were serially diluted, and an appropriate dilution was counted using a hemocytometer. A 10 µL aliquot of the occlusion body suspension was loaded into the chamber by capillary action, and occlusion bodies were enumerated in five large squares (0.2 × 0.2 mm), with all 16 sub-squares

counted for calculation. The data obtained were then extracted to construct the qPCR standard curve. 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. Target gene sequencing and re-confirmation

Viral DNA was extracted using QIAamp MinElute Virus Kit, yielding high-quality DNA suitable for downstream molecular analyses. DNA *sequencing* is performed to confirm that the virus strain provided in the reference material is SeMNPV. The extracted DNA was further purified using the ExoSAP-IT PCR Product Cleanup Reagent before sequencing. Sanger sequencing was performed following PCR amplification of the target gene, and 10 µL of purified sequencing products were loaded into a 96-well plate and analyzed on a SeqStudio 3500 genetic analyzer using the MediumSeq module. The primers used to sequence the target gene are the Forward primer: 5'-AAC CAA AGT TGA CTC TCT TCA AGG AAAT-3' and Reverse primer: 5'-CCT ACG TAA ACG ATG GGT TTG TAA AAG TT-3' [11]. The obtained sequences confirmed the identity of the target region and were consistent with reference sequences of SeMNPV.

2.3.3. qPCR assay

The qPCR assay was designed to target the DNA polymerase (*dnapol*) gene of SeMNPV, a highly conserved genomic region widely used for virus identification and quantification. Amplification was performed using a primer pair specific to the SeMNPV DNA polymerase gene, including qDNApol-F: 5’-CCG CTC GCC AAC TAC ATT AC-3’ and qDNApol-R: 5’-GAA TCC GTG TCG CCG TAT ATC-3’ [12]. The qPCR reactions consisted of 5 µL Luminaris HiGreen qPCR master mix (2X), 10 µM of each primer, template DNA (100 - 200 ng), and water, reaching a total volume of 10 µL. The thermal cycling conditions included an initial denaturation at 95°C for 10 mins, followed by 40 cycles of denaturation at 95°C for 30 s and annealing at 62°C for 1 min, with the melting curve set up at 95°C for 15 s and 60°C for 10 for 15 s.

2.3.4. Method validation

Method validation was performed in accordance with ISO 22118:2011 and the Guidelines on performance criteria and validation of methods for the detection, identification, and quantification of specific DNA sequences and proteins in foods. The evaluated validation parameters included the limit of detection (LOD), accuracy (AC), specificity (SP), sensitivity (SE), limit of quantification (LOQ), repeatability (RSDr), and reproducibility (RSDR). LOD was defined as the lowest concentration of SeNPV that could be reliably detected and was determined by testing serial ten-fold dilutions followed by confirmation using ten independent replicate assays at the lowest detectable concentration. A concentration was accepted as the LOD when ≥ 90% of replicates produced positive amplification results.

AC, SP, and SE were assessed using target and non-target viral DNA samples. AC was calculated as the proportion of correct results among all tested samples, SP as the ability to correctly identify non-SeNPV samples, and SE as the ability to correctly detect SeNPV-positive samples. These parameters were determined based on true positive, true negative, false positive, and false negative results, with acceptance criteria of ≥ 90% for each.

LOQ was defined as the lowest concentration at which SeNPV could be quantified with acceptable precision and was evaluated by performing ten independent replicate assays at the lowest detectable concentration. A concentration was accepted as the LOQ only when all replicates yielded positive amplification with acceptable variability.

RSDr was evaluated by duplicate analyses with five replicates per sample under identical conditions (same analyst, equipment, reagents, and day) and expressed as relative standard deviation, with an acceptance limit of ≤ 0.25. RSDR was assessed by two independent analysts performing replicate measurements across different runs and expressed as relative standard deviation of reproducibility, with an acceptance criterion of ≤ 0.35.

2.3.5. Data analysis

Experimental results were processed and analyzed using the proprietary software integrated with the qPCR system, and further compiled and analyzed using Microsoft Excel to generate detailed datasets and support data interpretation.

3. RESULTS AND DISCUSSIONS

3.1. SeMNPV hemocytometer-based quantification

After purification with 0.1% SDS, an appropriate volume of the reference material was loaded into a hemocytometer chamber for OBs quantification. Counting was performed in triplicate, and the calculated results were recorded in **Table 1**.

Table 1. *Number of observed OBs at 5 large squares of the chamber*

Trial	1	2	3	4	5	SD	Mean
1	101	97	105	110	105	4.4	103.6
2	107	113	109	121	123	6.4	114.6
3	96	93	106	100	99	4.4	99
			Total				**105.7**

With a dilution factor of 10^3, the mean OBs concentration obtained from three independent counts was approximately 2.7×10^{10} OBs/mL, which is consistent with the manufacturer's statement of the reference material. The low standard deviation values across all trials indicate minimal dispersion among counts from the five large squares, demonstrating good uniformity of particle distribution and reliable precision of the hemocytometer counting method.

For SeMNPV, accurate quantification through plasmid intermediates and transformation-based approaches is not feasible due to the inherent structural characteristics of the virus. In contrast to the study previously reported by Ha *et al.,* SeMNPV exhibits a multigenomic nature, with multiple nucleocapsids simultaneously encapsulated within a single occlusion body [13]. As a result, cloning viral gene fragments into competent cells does not adequately represent the true multigenomic composition of the virus. Although hemocytometer-based counting is subject to operator-dependent variability, the good agreement observed in this study demonstrates its practical utility for SeMNPV quantification. Accordingly, the OBs concentration determined by hemocytometer counting was used as the reference input value for subsequent qPCR method development and validation, providing a simple and pragmatic approach for controlling OBs input levels during assay validation.

3.2. Reference material re-confirmation

Purified viral DNA with high quality, as indicated by an A260/280 ratio ranging from 1.8 to 2.0, was used as the template for gene sequencing analysis. Based on the primer pair employed, a target gene fragment with a length of 437 bp was successfully amplified and sequenced.

The obtained sequence was subsequently compared with available SeMNPV genome sequences in the NCBI database. Sequence alignment analysis revealed a high level of similarity (99%) with *S. exigua* multiple nucleopolyhedrovirus VT-SeAl1 (GenBank accession no. HG425343.1), which was originally isolated in Europe [14]. **Figure 1** presents the comparative analysis between the gene sequence generated in this study and the corresponding reference sequence reported in Europe, thereby confirming the identity and suitability of the reference material for qPCR method development and validation.

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Spodoptera exigua multiple nucleopolyhedrovirus isolate VT-SeAl1, complete genome
Sequence ID: HG425343.1  Length: 135653  Number of Matches: 1

Range 1: 199 to 635  GenBank  Graphics          ▼ Next Match  ▲ Previous Match

Score           Expect   Identities      Gaps        Strand
802 bits(434)   0.0      436/437(99%)    0/437(0%)   Plus/Plus

Query  1    AACCAAAAGTTGACTCTCTTCAAGGAAATTCGTATCGTCAAACCGGACACGATGAAACTG  60
            |||||||||||||||||||||||||||||||||||||||||||| |||||||||||||||
Sbjct  199  AACCAAAAGTTGACTCTCTTCAAGGAAATTCGTATCGTCAAACCAGACACGATGAAACTG  258

Query  61   GTCGTCAACTGGAGCGGCAAAGAGTTTCTCCGCGAAACTTGGACGCGTTTCATGGAAGAC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  259  GTCGTCAACTGGAGCGGCAAAGAGTTTCTCCGCGAAACTTGGACGCGTTTCATGGAAGAC  318

Query  121  AGCTTTCCCATCGTCAACGATCAAGAAATCATGGACGTTTTCCTCGTAATCAACATGAGA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  319  AGCTTTCCCATCGTCAACGATCAAGAAATCATGGACGTTTTCCTCGTAATCAACATGAGA  378

Query  181  CCAACGAGACCTAACCGTTGCTTCCGATTTTTGGCTCAGCACGCTCTCCGTTGCGATCCC  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  379  CCAACGAGACCTAACCGTTGCTTCCGATTTTTGGCTCAGCACGCTCTCCGTTGCGATCCC  438

Query  241  GACTACGTTCCTCACGAAGTCATCCGCATCGTCGAGCCCGTGTACGTCGGCACCAACAAC  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  439  GACTACGTTCCTCACGAAGTCATCCGCATCGTCGAGCCCGTGTACGTCGGCACCAACAAC  498

Query  301  GAATACCGCATCAGTTTAGCCAAAAAGGGCGGCGGTTGCCCCGTCATGAATCTCCACTCC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  499  GAATACCGCATCAGTTTAGCCAAAAAGGGCGGCGGTTGCCCCGTCATGAATCTCCACTCC  558

Query  361  GAGTACACCAACTCGTTCGAGGAATTCATCAACCGCGTCATTTGGGAGAACTTTTACAAA  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  559  GAGTACACCAACTCGTTCGAGGAATTCATCAACCGCGTCATTTGGGAGAACTTTTACAAA  618

Query  421  CCCATCGTTTACGTAGG  437
            |||||||||||||||||
Sbjct  619  CCCATCGTTTACGTAGG  635
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***Figure 1**. Comparison of the resulting genome and reference sequence on NCBI*

3.3. qPCR amplification

Based on the confirmation of viral identity through sequencing of the dnapol gene, the performance of the primer and thermal cycling conditions was subsequently evaluated. The experimental results are presented in **Figure 2**.

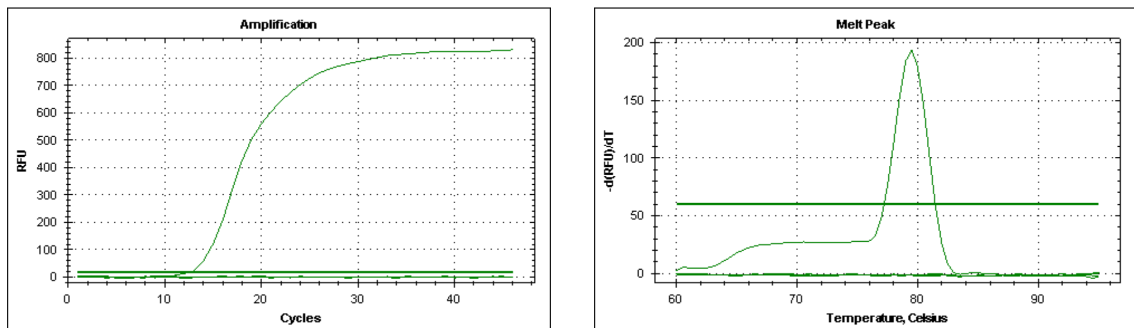


***Figure 2**. Amplification signal of the dnapol gene of SeMNPV (left) and corresponding melting curve (right)*

Evaluation of the primer and thermal profile produced clear, sigmoidal fluorescence amplification curve, indicating successful amplification of the target gene under the selected conditions. No amplification signal was observed in the negative control, confirming that the reaction was free from contamination and nonspecific amplification. The Ct ≈ 13.10 obtained in **Figure 2** (left) provides evidence of the high sensitivity of the developed qPCR assay. Melting curve analysis (**Figure 2**, right) showed a single, specific peak at 79.5°C, confirming the specificity of the amplified product. Based on these results, this DNA sample was subsequently used as the positive control for further experiments in the study.

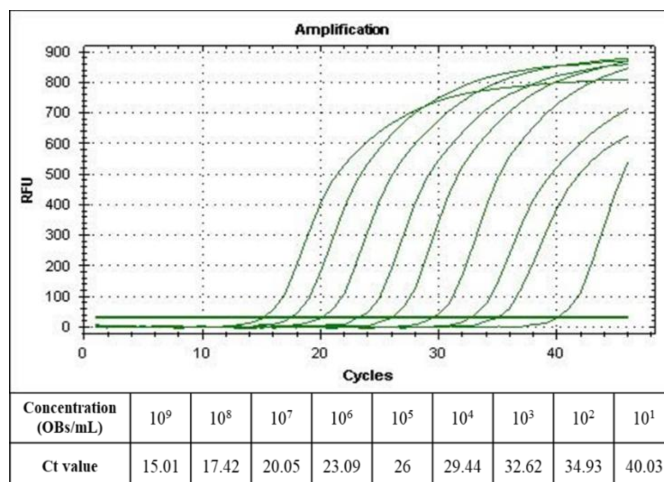
3.4. Method validation

3.4.1. Limit of detection (LOD)

LOD defines the lowest concentration of target analyte that can be reliably detected by an analytical method, providing a critical measure of assay sensitivity for low-level quantification. **Figure 3** illustrates the amplification and melting curve profiles obtained from serial ten-fold dilutions of SeMNPV DNA, ranging from 10^9 to 10^1 OBs/mL.

Figure 3 shows a clear, sigmoidal fluorescence amplification curves in increase for concentrations from 10^9 to 10^2 OBs/mL, with Ct values progressively shifting to higher cycle numbers as viral concentration decreases. This trend indicates a strong inverse relationship between template concentration and Ct value,

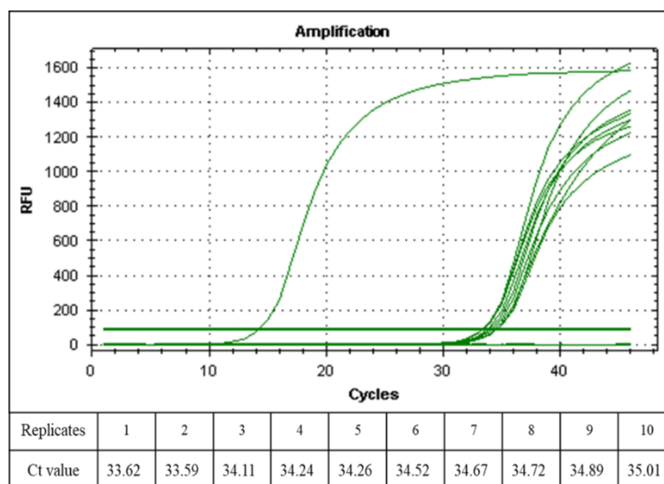
reflecting consistent amplification efficiency across the dynamic range tested. At the concentration of 10^1 OBs/mL, late amplification signals appearing at Ct values close to 40 were considered unreliable and non-reproducible, and therefore were not regarded as true positive amplification.



Concentration (OBs/mL)	10^9	10^8	10^7	10^6	10^5	10^4	10^3	10^2	10^1
Ct value	15.01	17.42	20.05	23.09	26	29.44	32.62	34.93	40.03

Figure 3. *Amplification signals at different concentrations (10^9-10^1 OBs/mL)*

As shown in **Figure 3** (left), the lowest concentration consistently producing a specific amplification signal with the correct melting temperature was 10^2 OBs/mL (Ct ≈ 34.93). Based on these results, the limit of detection (LOD) of the qPCR method was determined to be 10^2 OBs/mL.



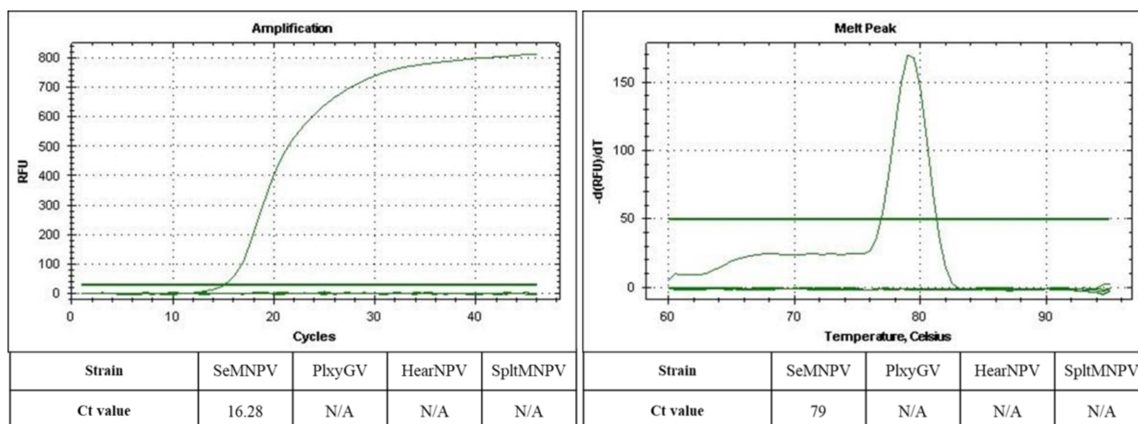
Replicates	1	2	3	4	5	6	7	8	9	10
Ct value	33.62	33.59	34.11	34.24	34.26	34.52	34.67	34.72	34.89	35.01

Figure 4. *Confirmation of the detection limit of the method*

To confirm the limit of detection, the lowest concentration was tested in 10 independent replicates. As shown in **Figure 4**, the positive control amplified early (Ct ≈ 13.95), while all 10 replicates at the lowest concentration produced detectable amplification (100% positive detection rate), supporting this concentration as the practical LOD of the assay. Since this study represents the first report describing the development of a qPCR assay for SeMNPV in Vietnam, the low limit of detection demonstrates the high analytical sensitivity of the method and its suitability for detecting low SeMNPV concentrations in plant protection products.

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

To evaluate accuracy (AC), specificity (SP), and sensitivity (SE), the amplification performance of the qPCR assay was assessed using two groups of targets: SeMNPV and non-SeMNPV viruses (including *Plutella xylostella* granulovirus PlxyGV, *Helicoverpa armigera* nucleopolyhedrovirus HearNPV, and *Spodoptera litura* multiple nucleopolyhedrovirus SpltMNPV).



Strain	SeMNPV	PlxyGV	HearNPV	SpltMNPV
Ct value	16.28	N/A	N/A	N/A

Strain	SeMNPV	PlxyGV	HearNPV	SpltMNPV
Ct value	79	N/A	N/A	N/A

***Figure 5**. Amplification signals in SeMNPV and non-SeMNPV groups (left) and corresponding melting curve (right)*

Figure 5 shows that a typical amplification signal (Ct ≈ 15.71) with a consistent melting temperature were observed exclusively in the sample containing SeMNPV DNA, characterized by a specific Ct value and a consistent melting temperature, whereas no amplification signals were detected from non-SeMNPV DNA samples. These results demonstrate that the qPCR assay achieved 100% accuracy, specificity, and sensitivity, fully complying with the performance defined by the applied validation standard.

3.4.3. Standard curve

The quantitative method was established based on the linear relationship between the target DNA concentration in the sample and the corresponding Ct values. Using the instrument's software, a standard curve was constructed as shown in **Figure 6.**

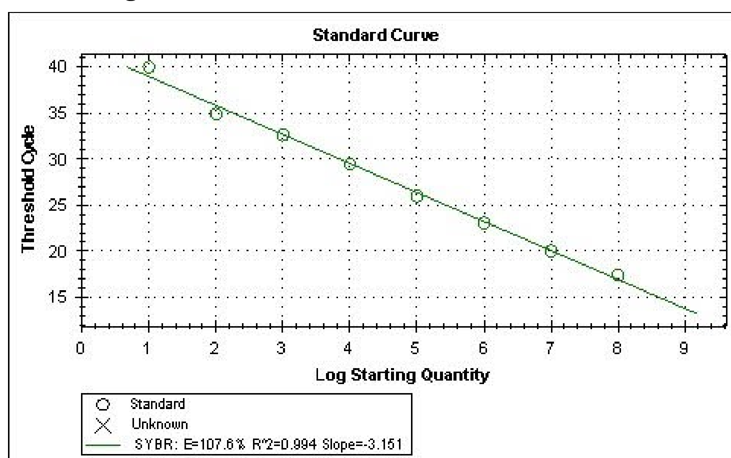
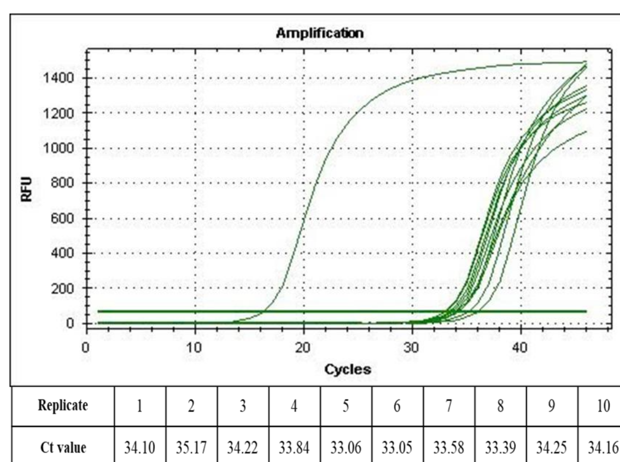


***Figure 6**. Standard curve of qPCR assay for SeMNPV*

The results demonstrated a strong linear correlation, with a coefficient of determination (R^2) equal to 0.994, a slope of -3.151, and an amplification efficiency (E) of 107.6%, which were all considered acceptable for qPCR [15]. The standard curve was described by a linear equation expressing the relationship between the threshold cycle (Y = Ct) and the base-10 logarithm of the initial target DNA copy number ($X = \log_{10}$ Sq), given as Y = -3.151X + 42.128. Based on this equation, the starting quantity of OBs was calculated using the formula Sq = 10^[(Ct - 42.128)/-3.151].

3.4.4. Limit of quantification (LOQ)

Based on the lowest detectable concentration, the limit of quantification of the method was evaluated by conducting 10 replicate assays using samples containing 10^2 OBs/mL (**Figure 7**).



Replicate	1	2	3	4	5	6	7	8	9	10
Ct value	34.10	35.17	34.22	33.84	33.06	33.05	33.58	33.39	34.25	34.16

***Figure** 7. Confirmation of the quantification limit of the method*

Figure 7 shows that all replicates produced positive amplification results, indicating a 100% detection rate and reliable quantification at this concentration. Accordingly, the LOQ of the method was established at 10^2 OBs/mL.

3.4.5. Repeatability (RSDr) and reproducibility (RSDR)

To evaluate repeatability, the experiments were performed in duplicate, with five replicates per sample. Reproducibility was assessed by having each analyst conduct 10 replicate measurements per sample. The results are presented in **Table 2**.

***Table** 2. Analysts' test results at the concentration of 102OB/mL*

Replicate	Analyst A - 1st time	Analyst A - 2nd time	Analyst B - 1st time	Analyst B - 2nd time
1	34.12	33.82	32.56	34.01
2	33.98	34.16	33.12	33.51
3	32.68	34.21	34.06	33.56
4	33.91	34.13	33.37	33.96
5	34.28	33.79	34.02	34.12

Based on the repeated Ct values obtained by the two analysts presented in **Table 2**, the RSDr values of the method for the two analysts (A and B) were calculated as 0.0045 and 0.0046, respectively. These values satisfy the acceptance criterion (≤ 0.25) in ISO 22118:2011, indicating acceptable standard deviation and good method stability. The RSDR of the method was determined to be 0.0048, which is below the specified limit of 0.35, demonstrating satisfactory precision of the method.

4. CONCLUSION

The study successfully developed a real-time PCR-based method for the detection and quantification of *Spodoptera exigua* multiple nucleopolyhedrovirus. The method exhibited high sensitivity, with both the limit of detection and the limit of quantification established at 10^2 CFU/mL, while specificity and accuracy reached 100%. The standard curve constructed using threshold cycle (Ct) values versus SeMNPV concentration showed excellent linearity (R^2 = 0.994) with a slope of -3.151. The repeatability, intermediate repeatability, and reproducibility standard deviations were 0.0045, 0.0046, and 0.0048, respectively, demonstrating good precision and accuracy of the method.

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REFERENCES

[1]. J. A. Powell and P. A. Opler, “Moths of western North America,” *Moths of Western North America*, 2009.

[2]. X. Wang *et al*., “Baculovirus Per Os Infectivity Factor Complex: Components and Assembly,” *Journal of Virology*, vol. 93, no. 6, 2019.

[3]. J. B. Reid, “Dibenzo- p -Dioxins: 2,3,7,8-Tetrachlorodibenzo- p -Dioxin,” *Patty’s Toxicology*, Apr. 2001.

[4]. T. K. Moekasan and R. S. Basuki, “Status Resistensi Spodoptera exigua Hubn. pada Tanaman Bawang Merah Asal Kabupaten Cirebon, Brebes, dan Tegal terhadap Insektisida yang Umum Digunakan Petani di Daerah Tersebut,” *Jurnal Hortikultura*, vol 17, no. 4, 2007 [in Indonesia].

[5]. D. M. Kolodny-Hirsch, T. Sitchawat, T. Jansiri, A. Chenrchaivachirakul, and U. Ketunuti, “Field evaluation of a commercial formulation of the Spodoptera exigua (Lepidoptera: Noctuidae) nuclear polyhedrosis virus for control of beet armyworm on vegetable crops in Thailand,” *Biocontrol Science and Technology*, vol. 7, no. 4, pp. 475-488, 1997.

[6]. M. A. Erlandson, U. Toprak, and D. D. Hegedus, “Role of the peritrophic matrix in insect-pathogen interactions,” *Journal of Insect Physiology*, vol. 117, 2019.

[7]. M. Liu *et al*., “Challenges of Cell Counting in Cell Therapy Products,” *Cell Transplant*, vol. 33, pp. 09636897241293628, 2024.

[8]. M. Arya, I. S. Shergill, M. Williamson, L. Gommersall, N. Arya, and H. R. H. Patel, “Basic principles of real-time quantitative PCR,” *Expert Review of Molecular Diagnostics*, vol. 5, no. 2, pp. 209-219, 2005.

[9]. P. M. de Andrade Zanotto and D. C. Krakauer, “Complete genome viral phylogenies suggest the concerted evolution of regulatory cores and accessory satellites,” *PLoS One*, vol. 3, no. 10, 2008.

[10]. I. Nazli-Huda, A. Sajap, and W. Lau, “Stability of Spodoptera litura nucleopolyhedrovirus in sodium dodecyl sulphate,” *African Journal of Biotechnology*, vol. 11, no. 16, pp. 3877-3881, 2012.

[11]. S. D. Woo, “Rapid detection of multiple nucleopolyhedroviruses using polymerase chain reaction,” *Molecules and Cells*, vol. 11, no. 3, pp. 334-340, 2001.

[12]. A. Carballo, R. Murillo, A. Jakubowska, S. Herrero, T. Williams, and P. Caballero, “Co-infection with iflaviruses influences the insecticidal properties of Spodoptera exigua multiple nucleopolyhedrovirus occlusion bodies: Implications for the production and biosecurity of baculovirus insecticides,” *PLoS One*, vol. 12, no. 5, 2017.

[13]. P. N. Ha, N. T. Thanh, H. A. Thu, T. Hong Ba, and V. T. Quy, “Development of a real-time PCR method for detection and enumeration of Plutella xylostella granulovirus in plant protection product,” *Vietnam Journal of Food Control*, vol. 8, no. 4, pp. 369-378, 2025.

[14]. J. Thézé, E. A. Herniou, O. Cabodevilla, L. Palma, P. Caballero, and T. Williams, “Genomic diversity in European Spodoptera exigua multiple nucleopolyhedrovirus isolates,” *Journal of General Virology*, vol. 95, no. Pt 10, pp. 2297-2309, 2014.

[15]. 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, p. 1261, 2015.