

Article

Highly Sensitive Fluorescent Detection of HPV-16 DNA Using Tungsten Disulfide Nanosheets and Exonuclease III-Assisted Signal Amplification

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Abstract

This study addresses the need for detecting human papillomavirus type 16 DNA (HPV-16), a high-risk factor for cervical cancer, by developing a highly sensitive fluorescence sensing method based on tungsten disulfide (WS₂) nanosheets and exonuclease III (EXO III)-assisted cyclic amplification. The method is constructed by combining the highly efficient fluorescence quenching capability of tungsten disulfide (WS₂) nanosheets with a fluorescein (FAM)-labeled complementary DNA (cDNA) probe. When the target HPV-16 is present, it specifically hybridizes with the cDNA to form a double-stranded structure. This double-stranded structure can be cleaved by EXO III. The cleaved cDNA is not adsorbed by WS₂ nanosheets, generating a significant fluorescence signal. The released HPV-16 can then participate in the reaction again, achieving multiple rounds of fluorescence signal amplification. Under optimal conditions, the detection limit of the method is 0.35 pM. The method was successfully applied to the detection of HPV-16 in spiked serum samples, demonstrating the advantages of operational simplicity, high sensitivity, and good specificity. It provides a promising rapid detection method for clinical application research related to human papillomavirus.

Keywords: HPV-16; WS₂ nanosheets; EXO III; fluorescence detection; serum

1. Introduction

Cervical cancer ranks among the most common malignant tumors in women worldwide, posing a serious threat to female health and life [1]. Epidemiological data indicate that the vast majority of cervical cancer cases are closely associated with persistent infection by high-risk human papillomavirus (HPV) [2]. Among the HPV subtypes, HPV-16 is recognized as one of the types with the highest carcinogenic risk, accounting for a significant proportion of all HPV-related cervical cancer cases [3]. HPV-16 infection is characterized by a long incubation period. Many infected individuals show no obvious clinical manifestations in the early stages, leading to delayed diagnosis and often missing the optimal window for intervention [4,5]. Therefore, the early and accurate detection of HPV-16 DNA has become a critical component in cervical cancer screening and prevention systems [6,7].



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Effective early detection can not only identify potential carriers, enabling risk stratification and timely intervention to significantly reduce the incidence and mortality of cervical cancer, but also provide crucial evidence for clinicians to assess disease progression and formulate personalized treatment plans.

With the development of molecular biology and sensing technologies, methods for detecting HPV-16 have become increasingly diversified [8–10]. Currently, commonly used clinical strategies mainly include nucleic acid testing, protein detection, and cytology combined with HPV testing [11,12]. Among these, nucleic acid testing technologies, represented by polymerase chain reaction (PCR), are currently the mainstream confirmatory methods due to their extremely high sensitivity and specificity, enabling direct detection of viral DNA and determination of viral load [13,14]. Protein detection focuses on early carcinogenic proteins expressed by the HPV virus, reflecting the activity state of the virus and its association with disease progression by detecting these specific proteins [15]. The combined screening model integrating cytological examination with HPV DNA testing has been proven to significantly improve the detection rate of cervical precancerous lesions, effectively reducing the missed diagnosis and misdiagnosis risks associated with single methods [16]. These mature technologies have made significant contributions to the early diagnosis and global control of cervical cancer. However, the existing technological systems still face numerous challenges when applied to large-scale population screening, especially in resource-limited settings. Firstly, nucleic acid amplification technologies represented by PCR typically rely on precise temperature control equipment, professional laboratory environments, and well-trained personnel, resulting in long detection cycles and high costs. Secondly, although protein detection avoids amplification steps, its sensitivity sometimes fails to meet the requirements for detecting early infections or samples with low viral load. Furthermore, cytological examination is limited by subjective interpretation differences and requires a high level of professional expertise [17].

In recent years, emerging detection platforms, such as diagnostic tools based on the CRISPR-Cas system, microfluidic chip technology, and novel nanomaterial sensors, offer new avenues for simplifying operations, improving portability, and reducing costs [18–20]. For example, the CRISPR-Cas12a system leverages its trans-cleavage activity to enable highly specific amplification-free detection [21]; and integrated microfluidic devices combine signal amplification and readout into one unit, greatly simplifying the operational process [22]. The utilization of two-dimensional nanomaterials, such as graphene oxide and molybdenum disulfide, which can efficiently quench the fluorescence of dye-labeled single-stranded DNA probes via mechanisms like Förster resonance energy transfer or π - π stacking interactions, also represents a promising approach for highly sensitive detection [23,24]. However, the synthesis methods for these nanomaterials are often complex, and commercially available nanomaterials tend to be expensive. Consequently, achieving an optimal balance among sensitivity, specificity, detection speed, cost, and operational simplicity remains a key challenge for these new technologies [25,26]. Thus, the development of a novel HPV-16 detection method that combines high sensitivity, high specificity, speed, affordability, and ease of operation to meet the urgent needs of clinical diagnosis and large-scale screening has become an important research direction in this field.

In this study, we developed a novel fluorescence sensing method for the highly sensitive detection of HPV-16. This method integrates the efficient fluorescence quenching properties of tungsten disulfide (WS₂) nanosheets with an enzyme-assisted cyclic amplification strategy employing Exonuclease III (EXO III) (Figure 1). A FAM-labeled complementary single-stranded DNA (cDNA) probe was designed, which can efficiently adsorb onto the surface of WS₂ nanosheets via π - π stacking, leading to fluorescence quenching

due to FRET. In the presence of HPV-16, it specifically hybridizes with the cDNA, forming a double-stranded DNA structure. This structure can be specifically recognized by EXO III, which progressively cleaves the cDNA from its 3' end, releasing FAM-labeled short fragments and the intact HPV-16. The released HPV-16 can then bind to other cDNA probes, triggering a new round of hybridization and enzymatic cleavage cycles. Simultaneously, the fluorescence of the released FAM-labeled short fragments is no longer quenched by the WS₂ nanosheets. Through this cascade “binding-cleavage-release” recycling reaction, the cyclic reuse of the target HPV-16 and significant amplification of the fluorescence signal are achieved. Ultimately, sensitive detection of HPV-16 DNA is realized by monitoring the recovered fluorescence intensity. This method offers advantages such as simple operation, high sensitivity, and good specificity, demonstrating promising application potential in the field of clinical molecular diagnosis.

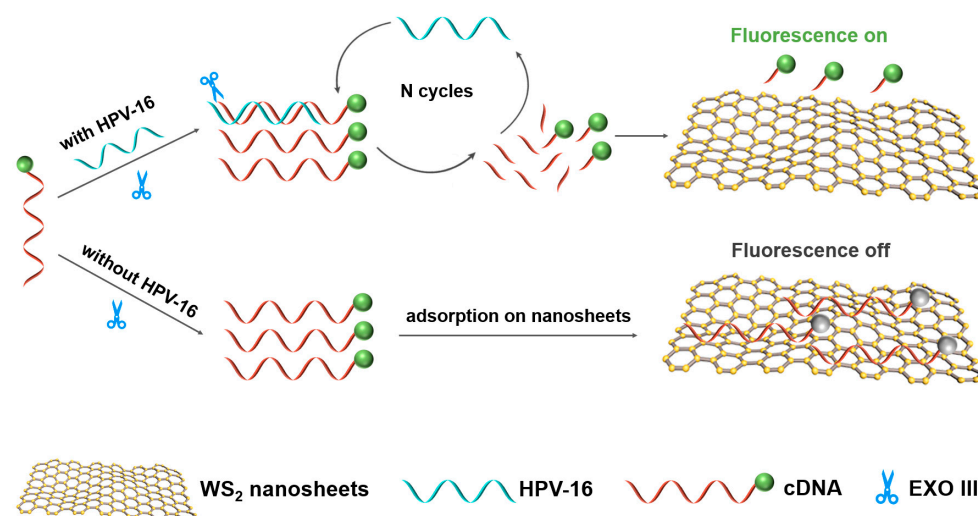


Figure 1. Schematic diagram of the fluorescent detection of HPV-16 based on WS₂ nanosheets and EXO III-assisted signal amplification.

2. Materials and Methods

2.1. Synthesis of WS₂ Nanosheets

WS₂ nanosheets were synthesized using the liquid-phase exfoliation method [27]. First, 2 g of WS₂ powder was dispersed in 20 mL of chitosan solution (0.8 mg mL^{−1}). The resulting mixture was transferred into a round-bottom flask. The mixture was sonicated for 12 h to obtain WS₂ nanosheets. The supernatant containing the exfoliated WS₂ nanosheets was collected after centrifugation at 3000 rpm for 20 min, and the unexfoliated precipitate at the bottom was discarded. Subsequently, the supernatant was centrifuged at 8000 rpm for 30 min and precipitate was collected. Finally, the WS₂ nanosheets were redispersed in ultrapure water. The effect of different sonication times on the synthesis of WS₂ nanosheets was investigated experimentally. The TEM image of WS₂ nanosheets was obtained using an accelerating voltage of 120 kV and a magnification of 120,000 $\times$.

2.2. Optimization of Conditions

First, the concentration of WS₂ nanosheets was optimized. Different concentrations of WS₂ nanosheets (0, 5, 10, 20, 30, 40, 50, 75, 100, 200, and 300 $\mu\text{g}\cdot\text{mL}^{-1}$) were added to 200 nM cDNA solution and incubated at room temperature for 15 min to determine the optimal quenching concentration. After the optimal concentration was determined, it was kept constant and the fluorescence spectra of cDNA and WS₂ nanosheets were measured after different reaction times (0, 5, 10, 15, 20, 30, and 40 min). Furthermore, to obtain the optimal EXO III concentration, different concentrations of EXO III (0.1, 0.2, 0.3, 0.5, 1.0,

2.0, and $3.0 \text{ U} \cdot \mu\text{L}^{-1}$) were added to the HPV-16/cDNA detection system and incubated at 37°C for 1 h. Finally, using the optimal concentrations of cDNA and EXO III, the reaction with 50 nM HPV-16 was carried out at 37°C for different times (0, 0.25 h, 0.5 h, 0.75 h, 1.0 h, 1.5 h, and 2 h). Subsequently, WS_2 nanosheets were added, and the fluorescence spectrum was measured after continuing the reaction at 37°C for another 15 min. Fluorescence emission was monitored from 500 to 650 nm under 480 nm excitation. The measurements were performed with a scan rate of 1200 nm/min and slit widths of 10 nm for both excitation and emission.

2.3. HPV-16 Detection

A mixture containing 40 μL cDNA ($2 \mu\text{M}$), 40 μL of HPV-16 at varying concentrations, 30 μL EXO III ($5 \text{ U} \cdot \mu\text{L}^{-1}$), 15 μL EXO III buffer ($10\times$), and 25 μL ultrapure water was prepared. The mixture was reacted at 37°C for 1 h. Subsequently, 20 μL WS_2 nanosheets ($4 \text{ mg} \cdot \text{mL}^{-1}$) and 230 μL TM buffer were added, followed by an additional 15 min of reaction. Fluorescence emission spectra were recorded from 500 to 650 nm under 480 nm excitation.

2.4. HPV-16 Detection in Serum Samples

To assess the practicality of our method in complex biological matrices, serum samples collected from healthy donors at Fujian Provincial Hospital were analyzed. Prior to detection, the samples were denatured by heating at 95°C for 10 min and then processed for HPV-16 detection according to the standard fluorescence assay protocol as described in Section 2.3.

3. Results

3.1. Characterization of WS_2 Nanosheets

We first evaluated the exfoliation efficiency of the WS_2 nanosheets. As shown in Figure 2A, the intensity of the characteristic absorption peak at 630 nm [28] gradually increased with prolonged sonication time. The absorption curves for 12 h and 15 h were essentially identical. Therefore, 12 h was chosen as the optimal sonication time. Transmission electron microscopy (TEM) images (Figure 2B) revealed that the bulk WS_2 was effectively exfoliated into thin-layer nanosheets, with sizes ranging approximately from 100 to 400 nm. The chemical composition of the WS_2 nanosheets was confirmed using X-ray photoelectron spectroscopy (XPS). The XPS spectra displayed peaks for the W $4f_{7/2}$ and W $4f_{5/2}$ at 32.4 eV and 34.6 eV, respectively (Figure 2C). Peaks for the S $2p_{3/2}$ and S $2p_{1/2}$ were observed at 162.3 eV and 163.5 eV (Figure 2D), respectively. These results are consistent with literature reports [29], confirming the successful synthesis of WS_2 nanosheets.

3.2. Feasibility Analysis and Condition Optimization

To enhance sensing performance, we first optimized the concentration of the WS_2 nanosheets, which are known to effectively adsorb DNA via van der Waals forces [30,31]. When the concentration of cDNA was kept constant, the fluorescence intensity of cDNA decreased with increasing concentration of WS_2 nanosheets (Figure 3A). When the concentration of WS_2 nanosheets increased to $200 \mu\text{g} \cdot \text{mL}^{-1}$, the fluorescence quenching efficiency reached 93%, demonstrating that WS_2 nanosheets are an effective quencher. Further increasing the WS_2 nanosheets concentration to $300 \mu\text{g} \cdot \text{mL}^{-1}$ did not cause a significant change in fluorescence intensity (Figure 3B). Therefore, a WS_2 nanosheets concentration of $200 \mu\text{g} \cdot \text{mL}^{-1}$ was selected. The WS_2 nanosheets exhibited better fluorescence quenching efficiency compared to the WS_2 powder (Figure S1). Next, we analyzed the effect of the reaction time between WS_2 nanosheets and cDNA on the fluorescence intensity of cDNA.

Figure 3C shows the fluorescence spectra of cDNA at different reaction times. The fluorescence curves gradually decreased over time. By comparing the changes in fluorescence intensity at 520 nm, a significant decrease was observed within 0–15 min, after which the intensity reached equilibrium (Figure 3D). Consequently, 15 min was chosen as the optimal reaction time between WS₂ nanosheets and cDNA.

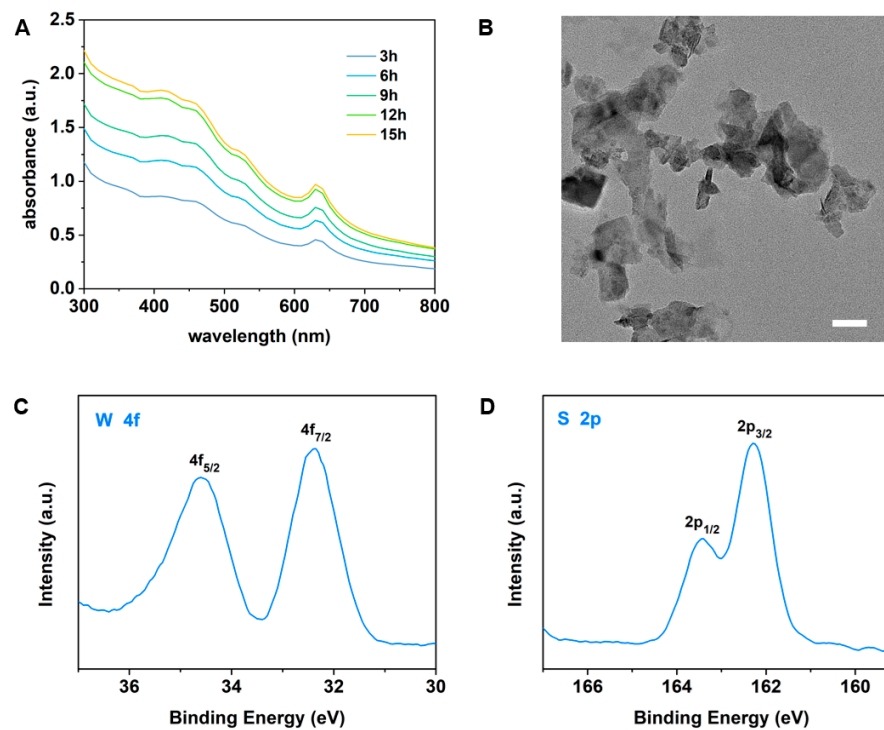


Figure 2. (A) UV-vis absorption spectra of WS₂ nanosheets prepared with different sonication times. (B) TEM image of WS₂ nanosheets (scale bars = 50 nm). XPS spectra of WS₂ nanosheets: (C) W 4f, and (D) S 2p electrons.

3.3. Verification of the EXO III-Assisted Signal Amplification Strategy

To verify the feasibility of the EXO III-mediated cyclic cleavage amplification strategy, we performed gel electrophoresis analysis using HPV-16 and cDNA as a model. Compared to the bands for HPV-16 alone (Figure 4A, lane 2) and cDNA alone (lane 3), a new band distinct from both appeared after incubating HPV-16 with cDNA, indicating the formation of HPV-16/cDNA double-stranded structure (lane 4). When EXO III was added to the HPV-16/cDNA double-stranded structure, it was observed that EXO III effectively cleaved the cDNA, causing the disappearance of the HPV-16/cDNA double-stranded structure band and the re-appearance of the HPV-16 band (lane 5). The released HPV-16 could then hybridize with another cDNA molecule, initiating the next round of hybridization and further amplifying the fluorescence signal of the detection system via EXO III activity. We further validated the feasibility of the fluorescence signal amplification detection using fluorescence spectroscopy. Figure 4B shows the fluorescence emission spectra of cDNA under different conditions. Compared to the intrinsic fluorescence of cDNA (Figure 4B, red line), when WS₂ nanosheets were introduced into the system, the strong van der Waals interaction between the cDNA bases and the WS₂ nanosheets surface caused cDNA adsorption. The broad absorption spectrum of WS₂ nanosheets highly overlaps with the fluorescence emission spectrum of cDNA, leading to fluorescence resonance energy transfer (FRET) and a significant decrease in cDNA fluorescence intensity (blue line). In the presence of HPV-16, efficient hybridization with cDNA formed HPV-16/cDNA double-stranded structure, which reduced the van der Waals interaction between cDNA and the

WS₂ nanosheets surface, causing cDNA to detach and resulting in fluorescence recovery (green line). Upon further addition of EXO III, the enzyme cleaved the cDNA within the double-stranded structure, releasing HPV-16 and the FAM fluorophore. The released HPV-16 could then hybridize with a new cDNA molecule, initiating the next cleavage cycle. Compared to the detection system without EXO III, this amplification strategy achieved a more significant fluorescence enhancement (yellow line). These results confirm the feasibility of the proposed fluorescence amplification method.

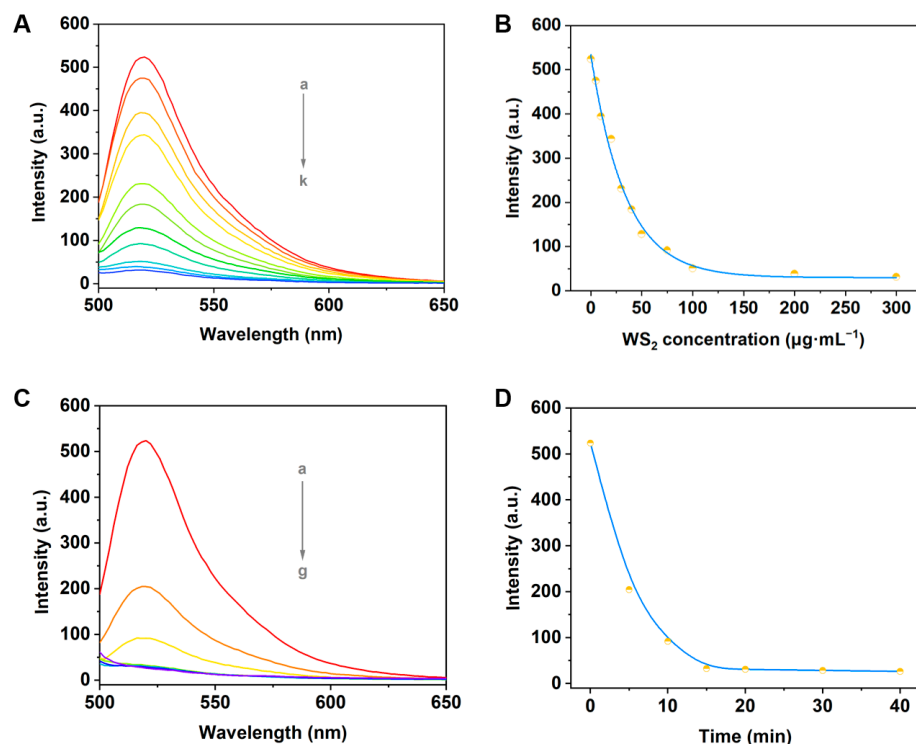


Figure 3. (A) Fluorescence spectra of cDNA after adding different concentrations (from a to k: 0, 5, 10, 20, 30, 40, 50, 75, 100, 200, and 300 µg·mL⁻¹) of WS₂ nanosheets. (B) Variation in fluorescence intensity of cDNA with WS₂ nanosheets concentration. (C) Fluorescence spectra of cDNA after incubation with WS₂ nanosheets at different times. (from a to g: 0, 5, 10, 15, 20, 30, and 40 min) of WS₂ nanosheets. (D) Variation in fluorescence intensity of cDNA with incubation times.

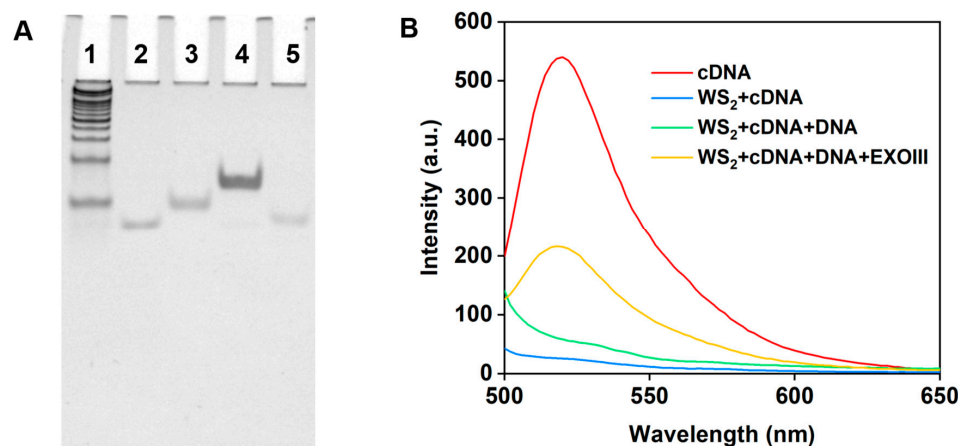


Figure 4. (A) Gel electrophoresis analysis for different samples. Lane 1: DNA marker; Lane 2: HPV-16; Lane 3: cDNA; Lane 4: HPV-16 + cDNA-16; lane 5: HPV-16 + cDNA-16 + EXO III. (B) Fluorescence spectra of cDNA in different cases.

3.4. Optimization of Detection Method

To improve the cleavage efficiency of cDNA, we optimized the concentration of EXO III. Different concentrations of EXO III (0.1, 0.2, 0.3, 0.5, 1.0, 2.0, and 3.0 U·μL⁻¹) were introduced into the detection system. The enzymatic cleavage efficiency was evaluated by measuring the increase in the fluorescence signal intensity of cDNA at these different EXO III concentrations. As shown in Figure S2A, the fluorescence spectra of cDNA intensified with increasing EXO III concentration. Notably, within the range of 0.1 to 1 U·μL⁻¹, the fluorescence signal increased significantly with enzyme concentration. However, when the EXO III concentration exceeded 1 U·μL⁻¹, the fluorescence signal value remained essentially constant (Figure S2B). Therefore, 1 U·μL⁻¹ was selected as the optimal EXO III concentration.

Furthermore, we optimized the reaction time and the cDNA concentration. First, the incubation time for HPV-16 with cDNA and EXO III was optimized (0, 0.25, 0.5, 0.75, 1.0, 1.5, and 2.0 h). As presented in Figure S3A, the fluorescence signal intensity increased with prolonged reaction time, but the increase became slight after 1 h (Figure S3B). Consequently, 1 h was selected as the optimal reaction time. Simultaneously, although reducing the amount of cDNA allowed the reaction system to reach equilibrium faster (Figure S4), the final fluorescence signal value of the detection system also decreased due to the lower cDNA concentration. Thus, 2 μM cDNA and a cleavage time of 1 h were chosen for the experiments.

3.5. HPV-16 Detection

The sensitivity of the fluorescence amplification method was evaluated by testing a series of HPV-16 concentrations (0, 0.001, 0.005, 0.01, 0.1, 1, 10, 25, 50, 75, 100, 200 nM) under the optimized conditions. As shown in Figure 5A, the fluorescence intensity of the detection system at 520 nm increased with the rising concentration of HPV-16, indicating that the method exhibits a high dependency on the target concentration for detecting HPV-16. Within the range of 0.001 to 1 nM, a good linear relationship was observed between the fluorescence intensity of the detection system and the concentration of HPV-16 (inset of Figure 5B). The linear regression equation was $F = 82.71 C_{\text{HPV-16}} + 15.61$ ($R^2 = 0.9947$), where $C_{\text{HPV-16}}$ represents the HPV-16 concentration and F represents the fluorescence intensity value of the detection system. The limit of detection was calculated to be 0.35 pM. Compared to alternative methods, our proposed fluorescent detection approach demonstrates competitive sensitivity, with a lower detection limit compared to several existing methods (Table 1).

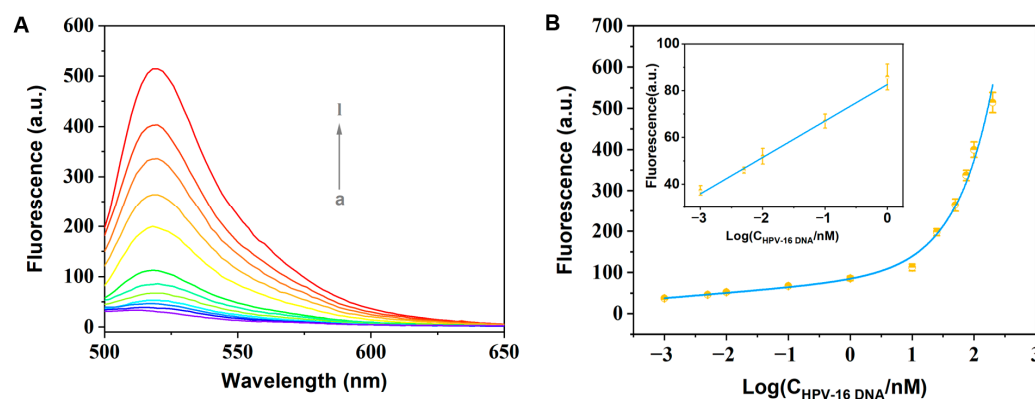


Figure 5. (A) Fluorescence spectra of the cDNA upon addition of HPV-16 at different concentrations (a–i: 0, 0.001, 0.005, 0.01, 0.1, 1, 10, 25, 50, 75, 100, 200 nM). (B) Standard calibration curve for HPV-16. Inset: Plot of fluorescence intensity at 520 nm versus HPV-16 concentration. The illustrated error bars represent the standard deviation of three repetitive measurements.

Table 1. Comparison of other reported HPV-16 detection methods.

Methods	Linear Range (nM)	LOD (pM)	Reference
Fluorescence	0.01–0.4	1.2	[32]
Fluorescence	1–1000	7.64	[33]
Fluorescence	0.005–0.05	5	[34]
Fluorescence	0.1–40	62	[35]
Colorimetric	0.001–1.0	1	[36]
Electrochemiluminescence	1–100	600	[37]
Fluorescence	0.001–1.0	0.35	This work

3.6. Specificity and Stability of the Method

The specificity of an analytical method is critical for ensuring accurate target detection. To comprehensively validate this aspect of our assay, we employed two independent validation strategies. First, we selected other DNA types, such as HPV-18, HPV-31, HPV-51, and HPV-58, as potential interferents. As shown in Figure 6A, compared to the addition of HPV-16, the fluorescence values were significantly low when HPV-18, HPV-31, HPV-51, or HPV-58 were introduced into the system ($p < 0.01$). This indicates that these interfering DNA strands do not hybridize with the cDNA, consequently preventing cDNA from being cleaved and degraded by EXO III and allowing it to remain adsorbed onto the WS₂ nanosheets. Furthermore, we added a negative control DNA sequence (control DNA) to the detection system and allowed it to react with different concentrations of HPV-16. Similarly, the fluorescence values of the detection system remained at a low baseline level and showed no correlation with the concentration of HPV-16, a trend that was statistically significant ($p < 0.01$, Figure 6B). The above results demonstrate that the designed fluorescence amplification method exhibits good specificity for HPV-16.

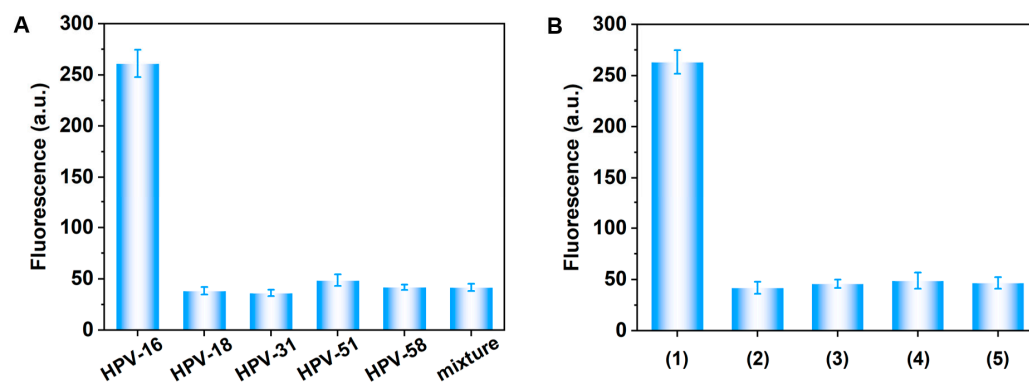


Figure 6. (A) Fluorescence intensity of the system toward target HPV-16 and other DNAs (mixture: HPV-18 + HPV-31 + HPV-51 + HPV-58). (B) Fluorescence intensity of different system: (1) cDNA + HPV-16 + EXO III + WS₂, (2) control DNA + HPV-16 (1 nM) + EXO III + WS₂, (3) control DNA + HPV-16 (10 nM) + EXO III + WS₂, (4) control DNA + HPV-16 (50 nM) + EXO III + WS₂ and (5) control DNA + HPV-16 (100 nM) + EXO III + WS₂. The illustrated error bars represent the standard deviation of three repetitive measurements.

The reproducibility of the WS₂ nanosheet is crucial for the practical application of this method in HPV-16 detection. As shown in Figure S5, three batches of WS₂ nanosheets were synthesized under identical conditions via the sonication method, and their detection performances were compared. The relative standard deviation (RSD) of the fluorescence signal changes generated by the three batches was 1.36%, indicating good reproducibility of the synthesized WS₂ nanosheets. We also investigated the stability of the WS₂ nanosheets, cDNA, and EXO III. By comparing the fluorescence quenching efficiency after different storage periods, it was found that the WS₂ nanosheets, cDNA, and EXO III retained

fluorescence quenching efficiencies of 93.3%, 98.3%, and 90.4%, respectively, after one month of storage (Figures S6–S8). These results suggest that the designed assay components possess good stability and are promising for practical applications.

3.7. Detection of HPV-16 in Serum Samples

To assess the feasibility of the proposed method for clinical analysis, we evaluated its performance using human serum samples. First, we compared the detection performance in buffer systems versus serum samples to evaluate potential matrix effects. The results showed no obvious matrix effects or significant fluorescence signal differences in the detection of HPV-16 between Tris-HCl buffer and serum solution (Figure S9). Next, we analyzed three individual clinical serum samples, all of which tested negative for HPV-16. To further evaluate the accuracy of the method in this complex matrix, these samples were spiked with known concentrations of HPV-16 (0.005, 0.01, and 0.1 nM) after a 10-fold dilution. As summarized in Table 2, the method demonstrated satisfactory recovery rates ranging from 96.0% to 112.4%, with relative standard deviations (RSDs, $n = 3$) between 2.26% and 4.89%. A comparative analysis with a standard method showed that the detection results were in close agreement. Collectively, these results indicate reliable analytical performance and support the potential utility of this method for detecting HPV-16 in real clinical specimens.

Table 2. Comparison of HPV-16 detection in serum samples between the proposed and standard methods.

Samples	Spiked Levels (nM)	The Developed Method			The qRT-PCR Method		
		Found (nM)	Recovery (%)	RSD (%)	Found (nM)	Recovery (%)	RSD (%)
Sample 1	0.005	0.00562	112.4	3.12	0.00503	100.6	2.95
	0.01	0.0104	104.0	3.72	0.0097	97.0	3.16
	0.1	0.0988	98.8	3.16	0.0956	95.6	2.40
Sample 2	0.005	0.00494	98.8	3.48	0.00521	104.2	2.62
	0.01	0.01024	102.4	4.27	0.00984	98.4	3.63
	0.1	0.1037	103.7	2.26	0.0943	94.3	3.37
Sample 3	0.005	0.0048	96.0	3.48	0.00491	98.2	2.62
	0.01	0.00983	98.3	2.63	0.01098	109.8	2.83
	0.1	0.0996	99.6	4.89	0.1102	110.2	3.52

4. Discussion

In this study, a novel and highly sensitive fluorescent method for HPV-16 DNA detection was successfully developed, leveraging the efficient fluorescence quenching capability of WS₂ nanosheets and an Exonuclease III (EXO III)-assisted cyclic amplification strategy. The method operates on a “binding-cleavage-release” signal amplification principle, where HPV-16 recycling leads to significant fluorescence recovery, enabling sensitive quantification. Under optimized conditions, the method achieved a low detection limit of 0.35 pM with a wide linear range and demonstrated excellent specificity against other HPV subtypes. Furthermore, the satisfactory recovery rates and precision obtained in spiked human serum samples confirm the method’s robustness and anti-interference capability in complex biological matrices. Compared to conventional techniques, this strategy integrates the advantages of nanomaterial-based sensing and enzymatic amplification, offering a promising alternative that is rapid, cost-effective, and operationally simple.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/bios16020111/s1>. Figure S1: Comparison of fluorescence changes using different quenching materials; Figure S2: Optimization of EXO III concentration; Figure S3: Optimization of the reaction time for the interaction HPV-16 with cDNA and EXO III; Figure S4: Optimization of cDNA concentration; Figure S5: The change in fluorescence intensity variations generated by three different batches of WS₂ nanosheets; Figure S6: The stability of WS₂ nanosheets; Figure S7: The stability of cDNA; Figure S8: The stability of EXO III; Figure S9: Comparison of fluorescence signal values in Tris-HCl buffer and serum; Table S1: Information about the oligonucleotides.

Author Contributions: Conceptualization, M.W. and G.L.; methodology, G.L.; software, M.W. and J.D.; validation, M.W. and G.L.; formal analysis, G.L. and J.D., A.Z. and H.H.; investigation, J.D., A.Z. and H.H.; resources, M.W. and J.D.; data curation, J.D., A.Z. and H.H.; writing—original draft preparation, M.W. and G.L.; writing—review and editing, Z.C. and C.H.; visualization, Z.C. and C.H.; supervision, Z.C. and C.H.; project administration, Z.C.; funding acquisition, Z.C. and C.H. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Dataset available on request from the authors.

Conflicts of Interest: The authors declare no conflicts of interest.

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