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參展科別　醫學與健康科學

作品名稱　**Fluorescent Nanodiamond Application in Dengue Fever Precision Detection (螢光奈米鑽石於登革熱精準檢測平台之應用與研究)**

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關鍵詞　**Dengue Fever (登革熱) 、Fluorescent Nanodiamond (螢光奈米鑽石)、Magnetic Modulation (磁場調控)**

作者簡介

我是陳柏諭，就讀台北市私立復興實驗高級中學雙語部的高中二年級。從小就喜歡閱讀環境科學及生物相關百科和雜誌，未來也計畫在大學參與相關的學習領域。藉由這次實驗，不只熟悉醫療與健康之領域，也多了解病毒是如何影響人們的生活，希望能對大眾和環境的健康有所貢獻。

我是任明威，就讀台北市私立復興實驗高級中學雙語部的高中二年級，平常喜歡用運動來保持身體健康。我對於日新月異的生物醫療科技很有興趣，尤其是在全球新冠疫情嚴重和病毒肆虐的時代，希望能協助研發出新穎及可商業化的病毒和疾病精準檢測技術平台，來保護人們的生命與健康。

摘要

登革熱是一種由蚊子傳播的全球性疾病，已在全球各國造成了嚴重破壞。當前沒有針對登革熱的疫苗或療法，使得早期診斷階對該疾病的預防極為重要。本實驗提出了一種新的診斷模型：自旋增強型側向免疫測定。自旋增強側流免疫分析平台與診斷標記物螢光奈米鑽石結合使用，利用電磁場調控螢光奈米鑽石發出的信號，以提供準確和靈敏的結果，取代傳統的奈米金側向免疫測定平台。我們發現基於螢光奈米鑽石的自旋增強型側面免疫分析法在登革熱病毒診斷中的應用不僅可以準確地檢測出登革熱病毒抗原 NS1，而且還提供了比基於奈米金的橫向流免疫分析法高約 100 倍的信號靈敏度。自旋增強型側向免疫測定法是針對登革熱病毒的一種改進的診斷工具，未來可以應用於其他病毒篩檢，例如寨卡病毒和新型冠狀病毒。

Dengue fever, a global disease that is transmitted by mosquitoes, has wreaked havoc in nations across the globe. Currently, there is **no vaccine or direct treatment** for Dengue fever, making the early **diagnostic** stage extremely essential to the prevention of the disease. In this experiment, a new model of diagnosis is proposed: **Spin-Enhanced Lateral Immunoassay** (SELFIA). Associating **lateral flow immunoassay** (LFIA) with an alternative diagnostic marker **Fluorescent Nanodiamond** (FND), spin-enhanced lateral immunoassay utilizes **electromagnetic fields** to modulate the signals from the nanodiamond's fluorescence and provides accurate and sensitive results, replacing the conventional but flawed diagnostic method of **colloidal gold lateral flow immunoassay**. We discovered the application of FND-based SELFIA on Dengue fever diagnosis not only accurately detects the Dengue virus antigen NS1, but it also provides signals that is approximately **100 times more sensitive** than those of colloidal gold-based LFIA. In conclusion, spin-enhanced lateral immunoassay is an improved diagnostic tool for Dengue virus, which can potentially be applied on other viruses such as Zika virus and Coronavirus in the near future.

壹、前言

一、研究動機

Dengue fever is a seasonal mosquito-borne disease and a prominent health issue in countries in the tropical and subtropical regions, including Taiwan as seen in Figure 1 [1]. Its number of infection cases has been rising in recent decades, with an estimated number of 50~100 million annual infections across the globe, putting almost one-third of the world’ s population at risk [2]. In the **2015 Dengue outbreak in southern Taiwan**, approximately 40,000 people were diagnosed and 218 of the patients died from the infection as seen in Figure 2 [3]. Without a vaccine or a direct treatment, Dengue infection plays an even bigger threat to our health [4]. However, the magnitude of the symptoms can be reduced with **early diagnosis** and actions. Currently, the majority of the world uses the diagnostic method of **gold nanoparticle lateral flow immunoassay** (LFIA), but since there had not been any major renewal since its first introduction in the 1980s, several drawbacks were reported by users, including insensitivity of detection that results in false-negative results [5]. Therefore, significant improvement to diagnostic accuracy is required in order to maximize the effect of early treatment. In this experiment, **Fluorescent Nanodiamond** (FND) is being tested as an alternative diagnostic marker along with the application of **magnetic modulation** of signals, proposing a new model for LFIA: **Spin-Enhanced Lateral Flow Immunoassay** (SELFIA) [6]. With its fluorescent and quantum spin properties, we seek to investigate the effectiveness of FND on LFIA and hope to contribute to the global prevention of viral infection [7].

二、研究背景

(一) Dengue Fever

Dengue fever is a tropical disease that causes symptoms such as high fever, headache, vomiting, muscle and joint pain, and skin rash [8]. It is caused by the Dengue virus, commonly carried and transmitted by mosquitoes in tropical and tropical regions such as Southeast and South Asia, Central America, South America, and Central Africa from Figure 1 [1]. Symptoms start to show at around 14 days after infection. Its danger is that many symptoms arise as simple flu symptoms, but when not taken care of, it could develop into hemorrhagic fevers and cause circulatory system damages [9]. There are around 400 million cases of Dengue fever each year and 96 million display sicknesses [2]. The virus has **four distinct serotypes** (DV1, DV2, DV3, DV4) with accumulative severity and contains the **non-structural protein antigen NS1**

[10]. Currently, there is no vaccine or cure to Dengue fever. Supportive treatments can only alleviate the symptoms. The only measures that are available to prevent Dengue Virus infections are to decrease mosquito populations and use mosquito repellent.

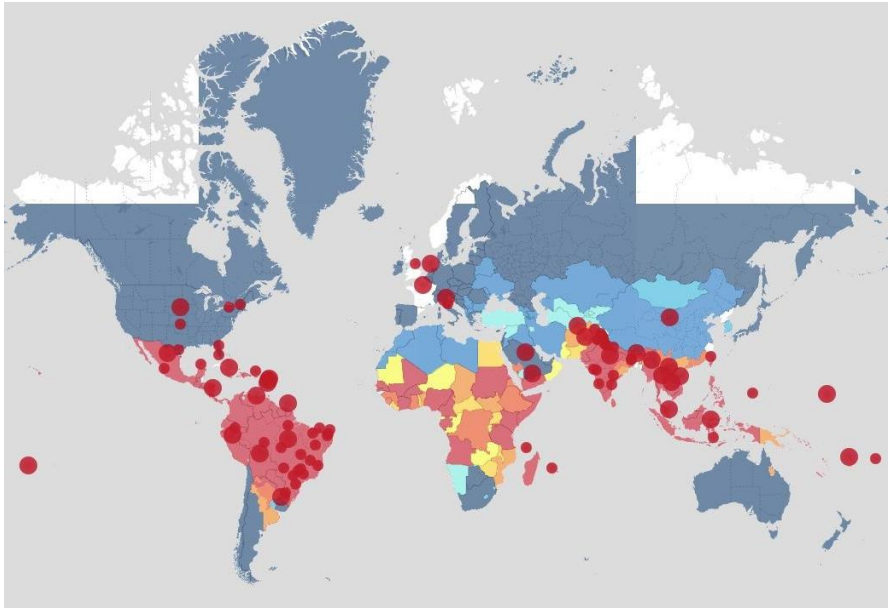


Figure 1. Distribution of Dengue Cases Worldwide. The location of Dengue fever risk worldwide is presented.

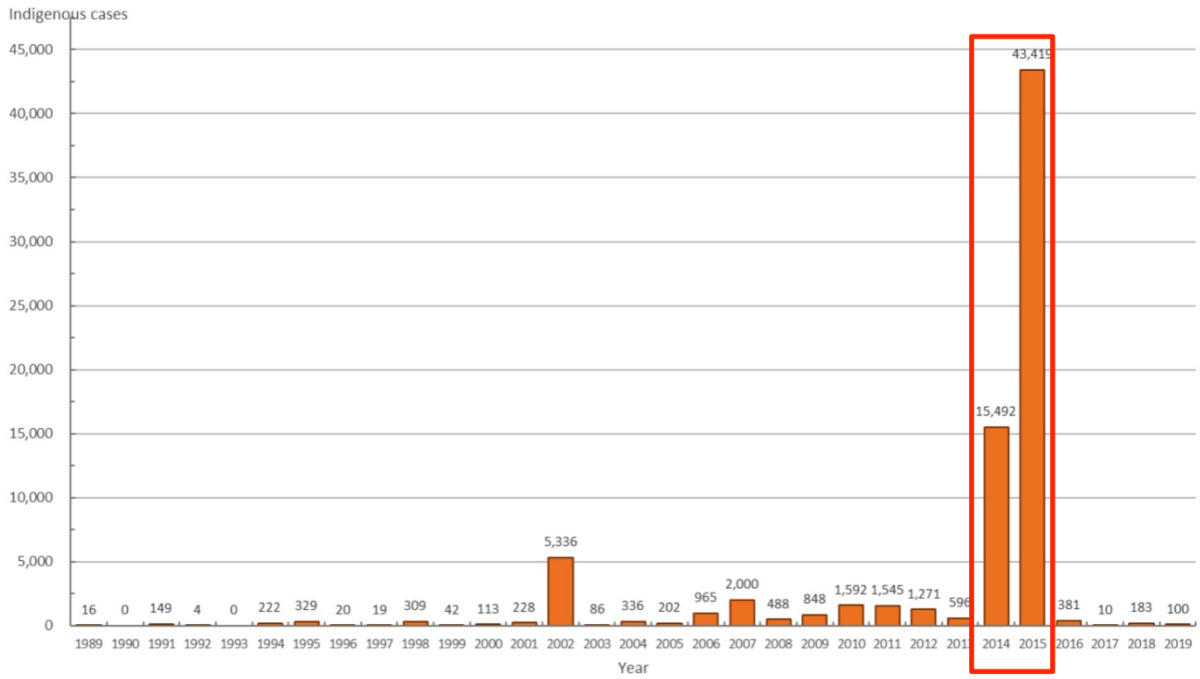


Figure 2. Number of Dengue Cases in Taiwan from 1989 to 2019. The chart shows the Taiwanese local cases of Dengue infection from 1989 to 2019. Source: Taiwan Centers for Disease Control: Dengue Fever.

(二) Lateral Flow Immunoassay

Lateral flow immunoassay (LFIA) is a **paper-based simple, instantaneous, and portable device**, specifically targeting the **detection of antigens/antibodies** [10]. Figure 3 shows the components of a LFIA: a test strip with a **sample pad** for the dispensation of biological samples, a **nitrocellulose membrane** where the substrates are immobilized and the **immunocomplexes** are formed, and an **absorbent pad** that provides the driving force of the liquid flow of **capillary action** and collects excess reagents filtered out by the nitrocellulose membrane [11-12]. A variety of liquid biological samples can be tested using LFIA, including urine, saliva, sweat, serum, plasma, whole blood, and other fluids through the principle of capillary action. The device is cheap and easy to use, making it a popular detection method among different applications from **blood tests** to **viral detection** [13-15]. Conventional method uses nanogold particle as a visual representation for most assays [16-17]. The method had not been updated for a while now.

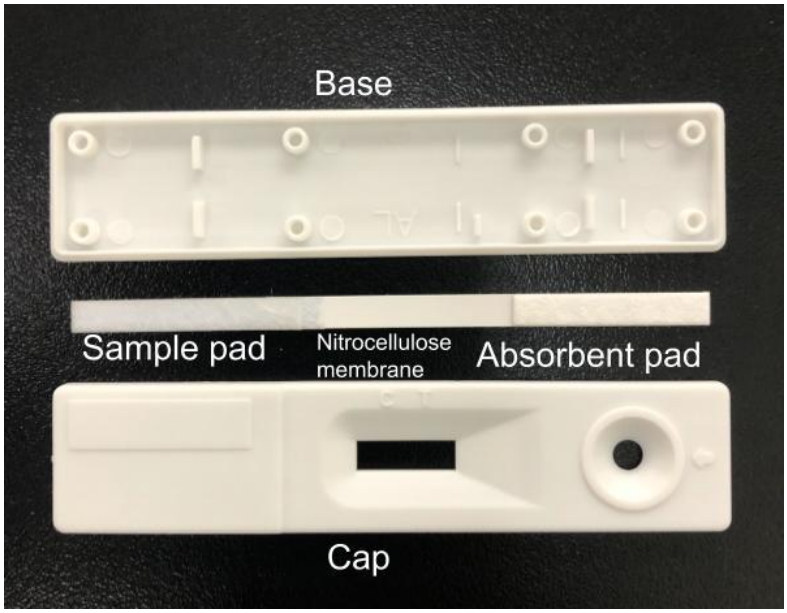


Figure 3. Lateral Flow Immunoassay Model. A model of LFIA's components with labels is shown in the picture.

(三) Fluorescent Nanodiamond

Fluorescent Nanodiamond (FND) is a novel nanoscale diamond developed by the Academia Sinica. Composed of sp3 bonded carbon atoms and nitrogen atoms, FNDs can work at a wavelength of 532nm and emit **red fluorescence** under laser excitation. FNDs can even be widely used in the fields of biomedicine and biotechnology due to several characteristics [6]: 1) they are highly **biocompatible** since the components of FNDs are common elements in living organisms; 2) due to the luminescence characteristics of its nitrogen-vacancy center (NV center), as seen in Figure 4, FNDs can emit **strong and long-lasting fluorescence** when excited; 3) because of its physical structure, FNDs can avoid the interference of background fluorescence and has a high degree of **specificity**; 4) the surfaces of FNDs can be **chemically modified** to contain emitters buried deep in a chemically inert matrix, so their optical properties are hardly affected by environmental changes (such as viscosity, pH, and ion concentration). Nowadays, FNDs has been studied for biological applications such as **nanoscale thermometry, drug delivery, and cell tracking** [18-25].

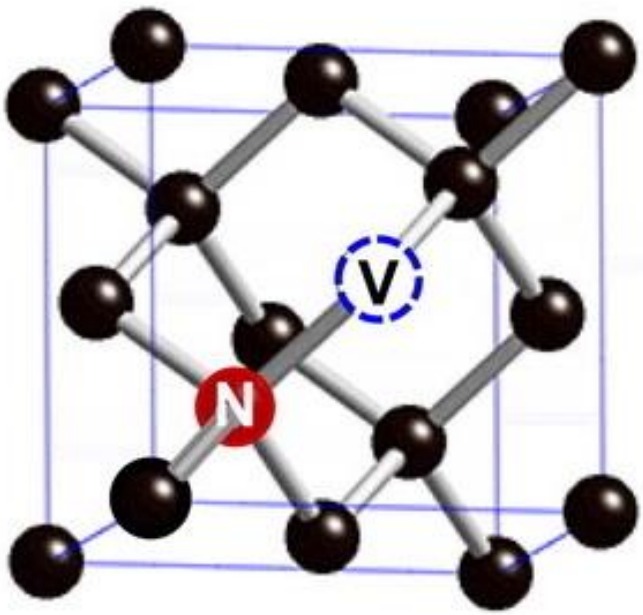


Figure 4. Fluorescent Nanodiamond. The diagram displays the structure of one single FND. It is composed of carbon and nitrogen atoms along with vacancy centers.

(四) Spin-Enhanced Lateral Flow Immunoassay

Spin-Enhanced Lateral Flow Immunoassay (SELFIA) is a system for measuring the levels of target antigens in biological samples on **lateral flow immunoassay** (LFIA) through the detection of **Fluorescent Nanodiamonds** (FND) [6]. Figure 5 displays the mechanism behind SELFIA. It utilizes a laser to excite the nitrogen-vacancy center ofFNDs and uses the fluorescence emitted as an indicator for LFIA, which performs antibody screening and antigen detection. The fluorescence of the diamond is first **modulated** by an **alternating current field** generated by a current amplifier and collected by the objective lens. The signals then travel to the photomultiplier tube. The electrical signals are then directed into a computer and analyzed by a LABVIEW program to achieve **ultra-sensitive detection and quantification of FNDs** [6]. Compared to conventional bioassays such as nanogold based lateral flow immunoassay, an improvement of the sensitivity over 100-folds can be established by using the magnetic modulation of FND’ s fluorescence and reduce false-negative results. It is an accurate, rapid, and accessible method for detecting infectious diseases [6].

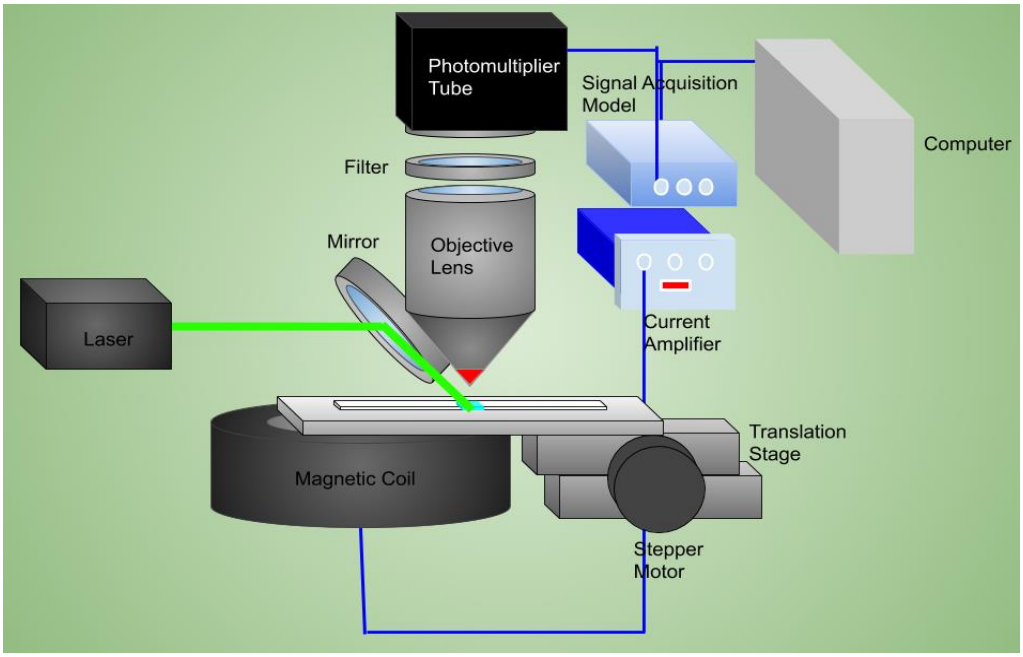


Figure 5. Spin-Enhanced Lateral Flow Immunoassay. A demonstration of the mechanism behind spin enhanced lateral flow immunoassay is shown.

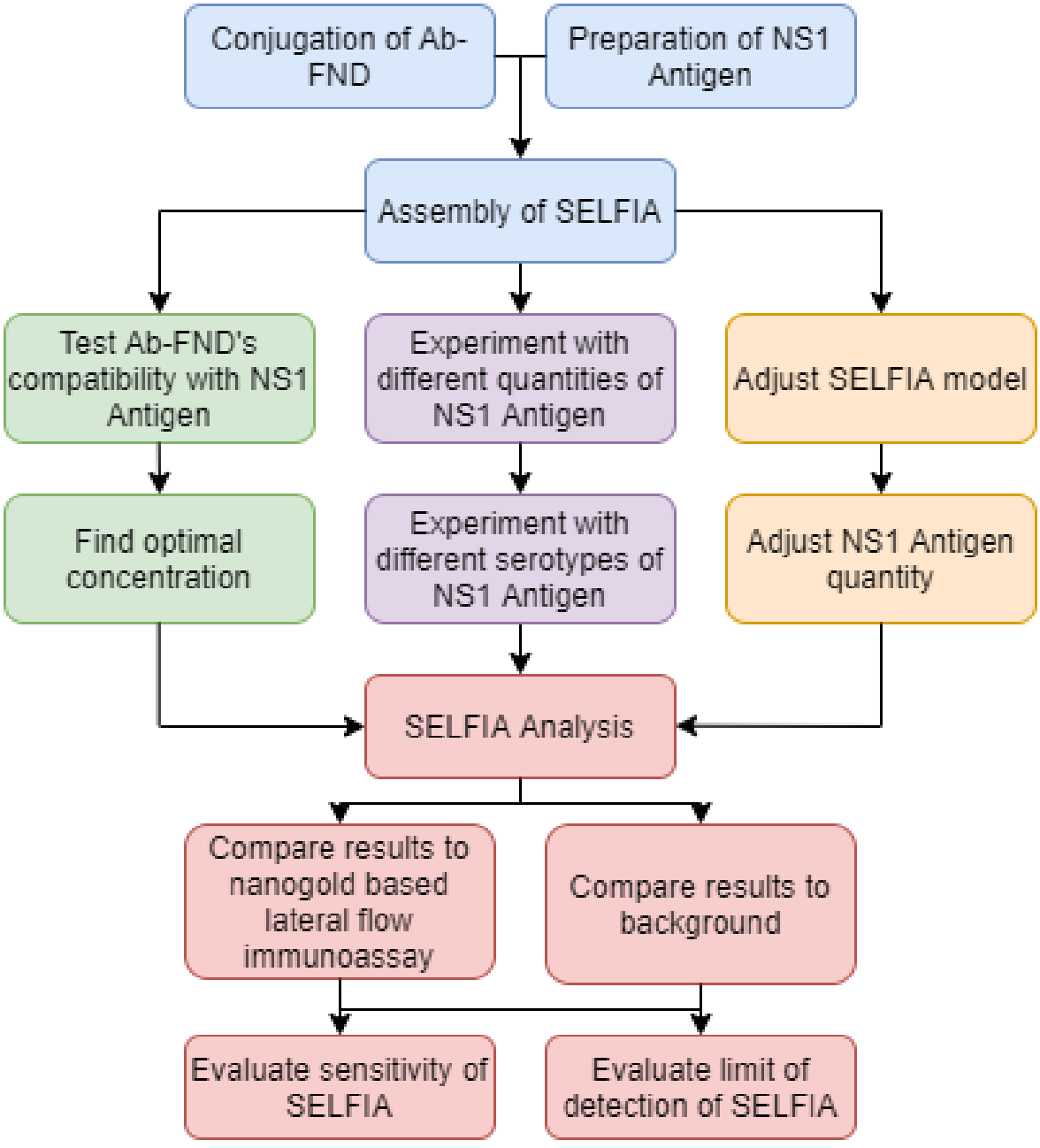
三、研究目的

(一) Test and compare the effectiveness of **nanogold-based lateral flow immunoassay** and **Spin-Enhanced Lateral Flow Immunoassay** on **Dengue NS1 antigen** detection.

(二) Explore a potential **diagnostic marker** for lateral flow immunoassay.

(三) Develop an **improved model** for lateral flow immunoassay for viral diagnosis.

貳、研究方法與過程



一、Reagents and Equipments

(一) Reagents

Fluorescent Nanodiamonds (FND) were produced by the laboratory of Prof. Huan-Cheng Chang at the Institute of Atomic and Molecular Sciences Academia Sinica (Taipei, Taiwan). Anti-Dengue virus 1+2+3+4 rabbit polyclonal antibody was purchased from Abcam (Cambridge, UK). NS1 antigen (DV1, DV2, DV3, DV4). Bovine Serum Albumin (BSA) was obtained from Sigma-Aldrich (St. Louis, MO, USA) Phosphate Buffered Saline (PBS) was purchased from Thermo Fisher Scientific (Waltham, MA, USA).

(二) Equipments

Centrifuge (Kubota, Osaka, Japan), ultrasonic bath (Cleanosonic, Richmond, VA, USA), lateral flow assay kit, spin-enhanced lateral flow immunoassay (SELFIA) (Institute of Atomic and Molecular Sciences Academia Sinica, Taipei, Taiwan), pipette, tube, oven.

二、Synthesis of Fluorescent Nanodiamonds

Figure 7 shows the stages of Fluorescent Nanodiamond production. The nanodiamond was first exposed to a hydrogen ion beam created by a particle accelerator. The central carbon atoms of the nanodiamond were removed, resulting in a vacancy center. Next, the nanodiamond was placed in a vacuum and heated at 800°C to move to join the nitrogen and vacancy together, forming a nitrogen-vacancy center that gave the nanodiamond the ability to deflect laser and emit fluorescence. Finally, the nanodiamond was oxidized at 450°C and washed with H2SO4:HNO3, removing the excess graphite and functionalizing the diamond’ s surface.

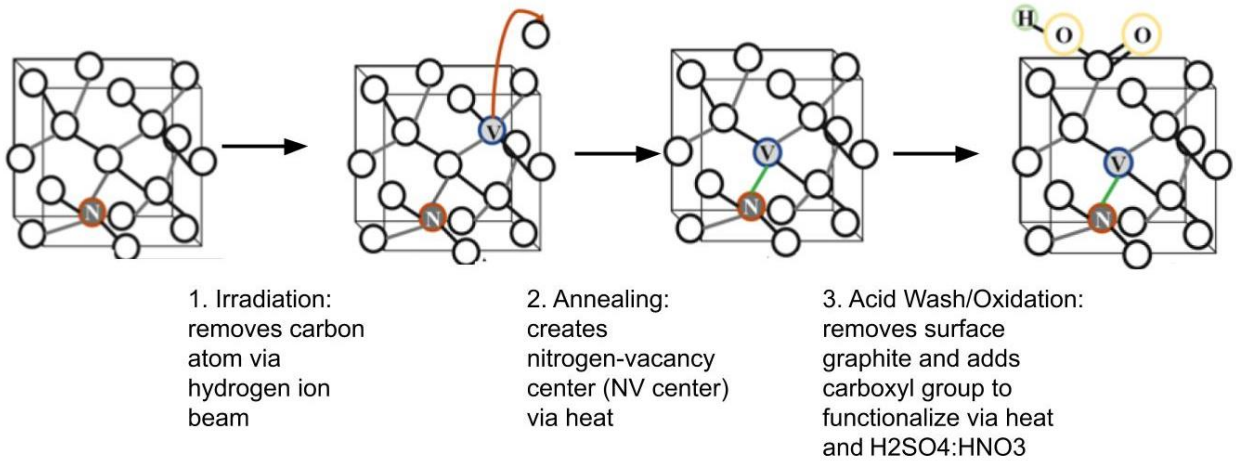


Figure 6. Process of Fluorescent Nanodiamond Synthesis. The production of FND is divided into three main steps: irradiation, annealing, and acid wash/oxidation.

三、Conjugation of Rb pAb-FND

FND (1μg/μL) was resuspended in ultrasonic bath before use. Anti-Dengue rabbit antibody (2μg/μL) was added. The tube was centrifuged at 20,000xg for 5 minutes to separate the unconjugated reagents from the mixture according to their size and density as shown in Figure 9. The supernatant was removed carefully. 100μl of 3% BSA in PBS was added. The solution was resuspended then pipetted 3 times to prevent the FNDs from cohering and ensures the even distribution of antibody on the FNDs surfaces as shown in Figure 8. Rb pAb-FND solution was then diluted to 75ng/μL (optimal concentration) with 3% BSA in PBS.

Figure 7. Ultrasonic Bath. The ultrasonic bath was used for the agitation of FNDs and the mixing of FNDs with anti-Dengue rabbit antibody.

Figure 8. Centrifuge. The centrifuge was used for the removal of excess supernatants after the conjugation of anti-Dengue rabbit antibody on FNDs.

四、Preparation of NS1 Antigen

The expression vector of the NS1 protein with different serum types (Type I, II, III, and IV) will be constructed into pcDNA3.4 vector for HEK293 cell line expression system. The large-scale production and purification of antigen samples (Dengue virus NS1 protein) will be carried out. The correct clones were determined by nucleotide sequencing and were transfected into suspension HEK293 cells. Suspension HEK293 Freestyle cells were grown in serum-free Freestyle 293 expression media at 37˚C, shaken at 110 rpm in an 8% CO2 incubator. These recombinant proteins will contain the C-terminal His-tag for further purification by Ni-NTA column.

五、Assembly of Lateral Flow Immunoassay

Lateral flow immunoassay kits were assembled as in Figure 10. NS1 DV1 (1.08 μg/μL), NS1 DV2 (1.89 μg/μL), NS1 DV3 (5 μg/μL), and NS1 DV4 (2.08 μg/μL) were diluted to 1μg/μL with 3% BSA in PBS. NS1 DV1, DV2, DV3, and DV4 (1μg/μL) were then diluted to 200ng/μL, 20ng/μL, and 2ng/μL with 3% BSA in PBS. 100ng and 10ng of NS1 DV1, DV2, DV3, and DV4 were dispensed onto the nitrocellulose membrane on LFIA and left to dry.

Re-optimization: Lateral flow immunoassay kits were assembled with the sample pads removed. NS1 DV1 (1.08 μg/μL), NS1 DV2 (1.89 μg/μL), NS1 DV3 (5 μg/μL), and NS1 DV4 (2.08 μg/μL) were diluted to 1μg/μL with 3% BSA in PBS. NS1 DV1, DV2, DV3, and DV4 (1μg/μL) were then diluted to 6.667ng/μL with 3% BSA in PBS. 100ng, 10ng, 1ng, and 0.1ng of NS1 DV1, DV2, DV3, and DV4 were dispensed onto the nitrocellulose membrane on LFIA and left to dry.

Figure 9. Lateral Flow Immunoassay Kit (assembled). The picture shows a LFIA kit in its complete form.

六、Run Rb pAb-FND on Lateral Flow Immunoassay

100μL of Rb pAb-FND (75ng/μL) was suspended onto the sample pad of the LFIA. The contraption was placed at room temperature for 30 minutes to run, then in oven at 50°C for 2 minutes to dry.

Re-optimization: 25μL of 3% BSA in PBS was aspirated onto the nitrocellulose to run as buffer. 100μL of Rb pAb-FND (75ng/μL) was then suspended. The contraption was placed at room temperature for 30 minutes to run, then in oven at 50°C for 2 minutes to dry.

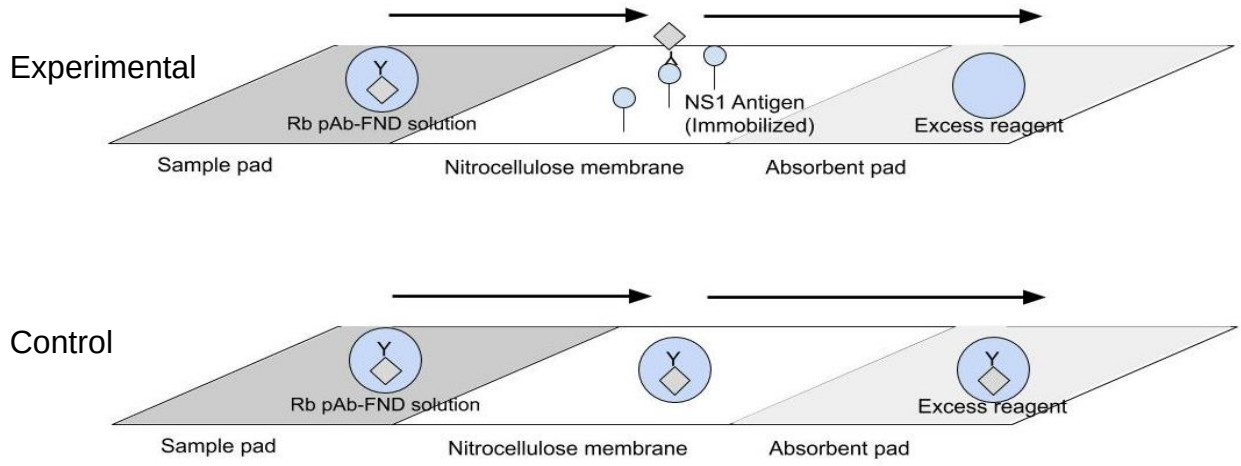


Figure 10. Demonstration of Lateral Flow Immunoassay Process. A simple schematic demonstration of the LFIA technique used in our experiment is presented.

Figure 10 shows the differences in mechanism in the experimental and control group. In the positive sample, the dispensed Rb pAb-FND solution associates with the immobilized NS1 antigens on the nitrocellulose membrane while the excess reagents are absorbed by the absorbent pad. In the negative sample, the dispensed Rb pAb-FND solution, instead of forming immunocomplexes with NS1 on the nitrocellulose membrane, flows directly to the absorbent pad due to the lack of NS1 antigen.

七、SELFIA Analysis

The finished samples were inserted into SELFIA. A laser beam was directed to the nitrocellulose membrane of the test strip. The test strip was placed on the platform and scanned as shown in Figure 11. As the FNDs on the test strip were excited by laser, fluorescence was emitted and absorbed by an objective lens, then transformed into electrical modulated signals that would be sent to a LABVIEW program to analyze the fluorescence of Rb pAb-FND.

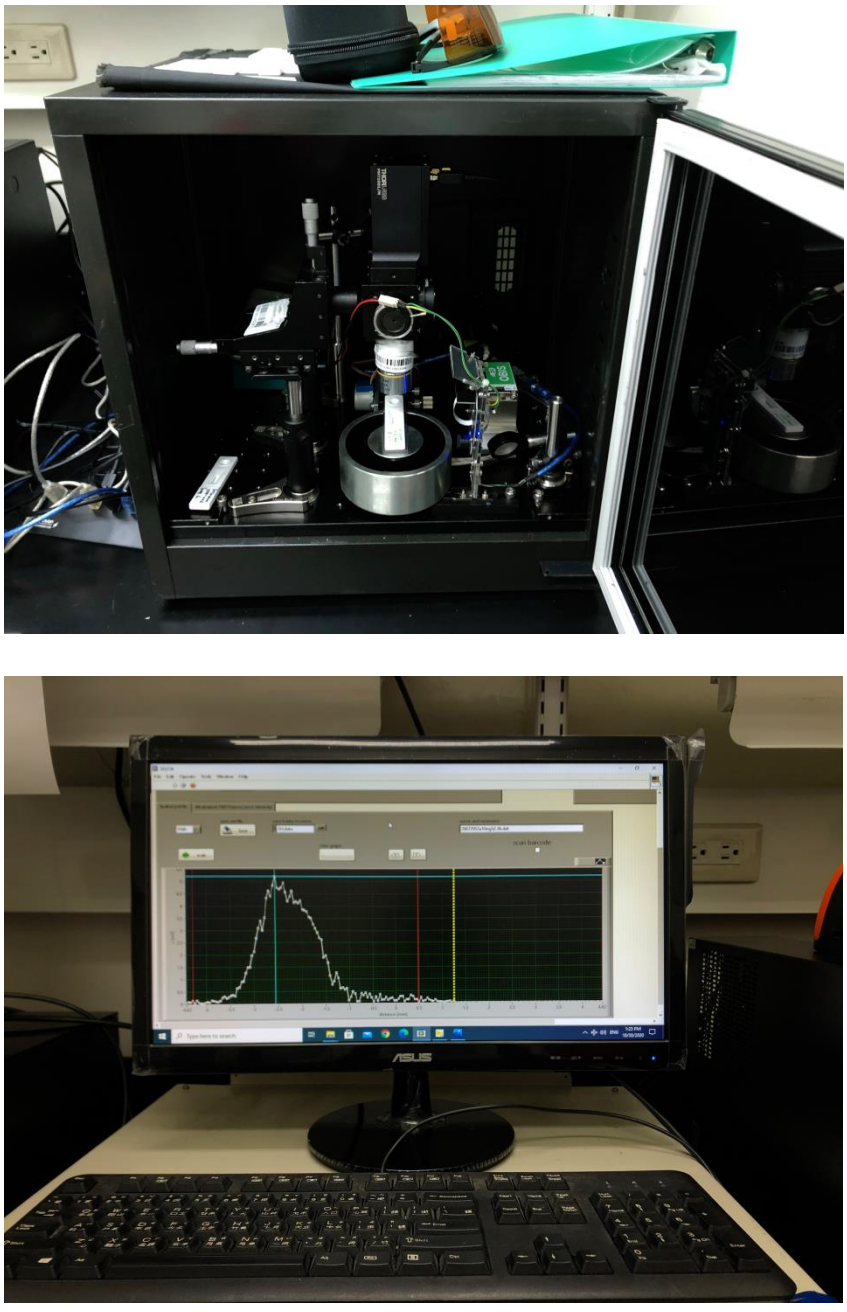


Figure 11. SELFIA. The pictures show the detection device for SELFIA.

參、研究結果與討論

一、Experimental Results

In this experiment, a new approach of combining LFIA and FND was tested. We first optimized the setup of the LFIA by finding the ideal concentration and ratio between the anti-Dengue rabbit antibody, the FNDs, and the NS1 antigen. We first applied and immobilized different serotypes of Dengue NS1 antigen on the membrane and dispensed Rb pAb-FND solution on to the test strip to observe the compatibility of different concentrations of Rb pAb-FND (50ng/μL, 75ng/μL, and 100ng/μL) with different NS1 types.

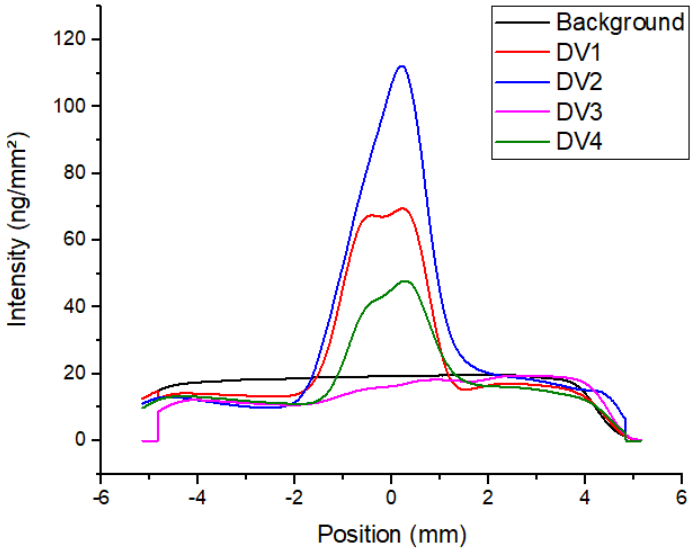


Figure 12. Results of 75ng/μL Rb pAb-FND on all four serotypes of NS1 lateral flow immunoassay. The graph shows the signal intensity detected on its relative positions on the LFIA test strips each with different serotypes of NS1 antigen immobilized.

In Figure 12, peaks in Rb pAb-FND mass distinct from the background/control group can be seen at the site of the dispensation of all four serotypes of NS1, which is a sign of the successful binding of Rb pAb-FND with all NS1 and accumulation of immunocomplexes at the designated point. This achieves the optimization as distinct peaks can be observed clearly and the background noises are relatively low.

Following the optimization stage, we evaluated the differences in intensity of signals by altering the quantity of NS1 antigen on the test strip. To do that, we added 100ng and 10ng of each type of NS1 onto the nitrocellulose membrane and ran Rb pAb-FND solution through the strip to observe the results.

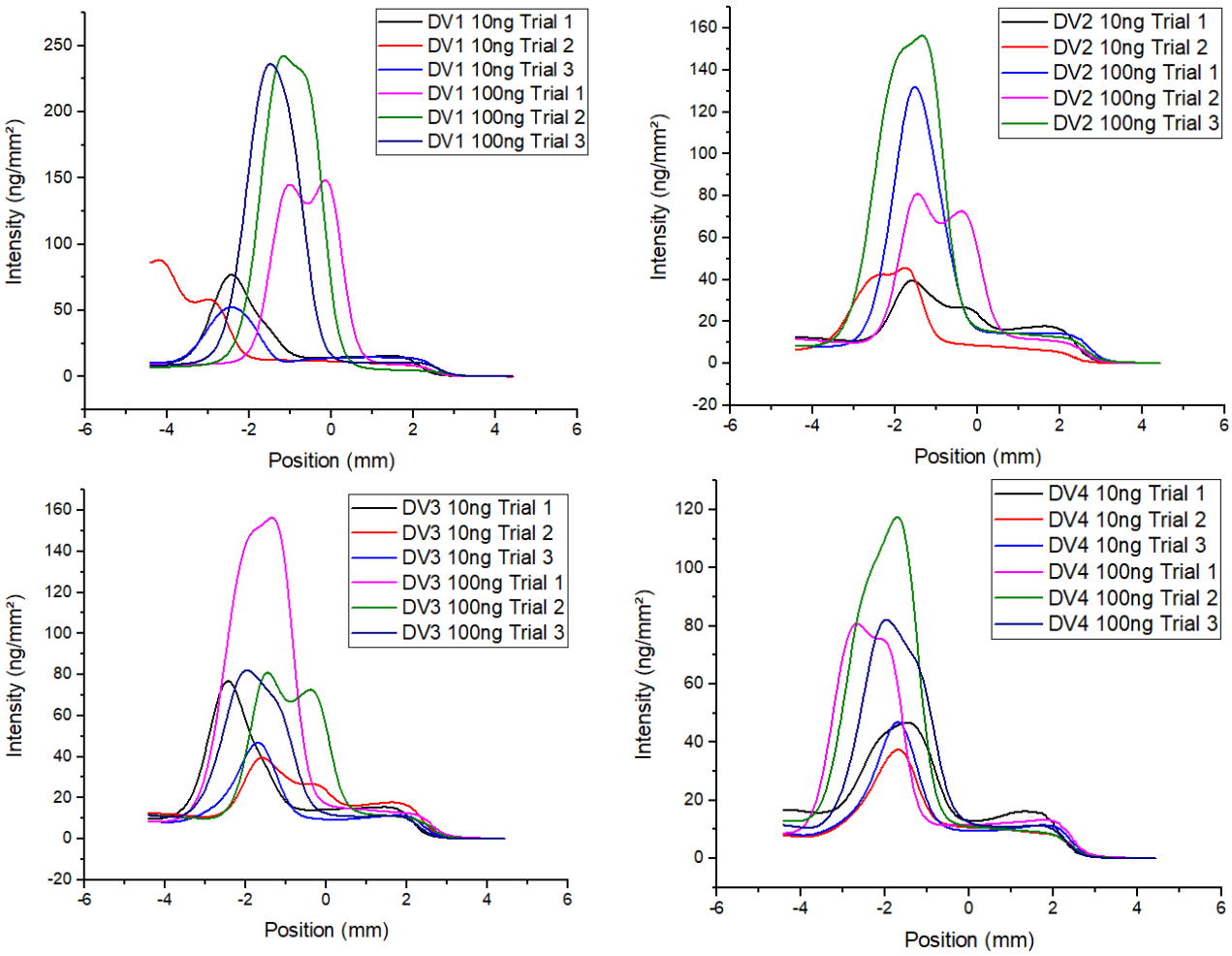


Figure 13. Results of 75ng/µL Rb pAb-FND on lateral flow immunoassay of 100ng and 10ng of all Dengue serotype NS1. The graph shows the comparison of signal intensity detected on its relative positions between LFIA test strips with 100ng and 10ng of all serotype NS1 antigen.

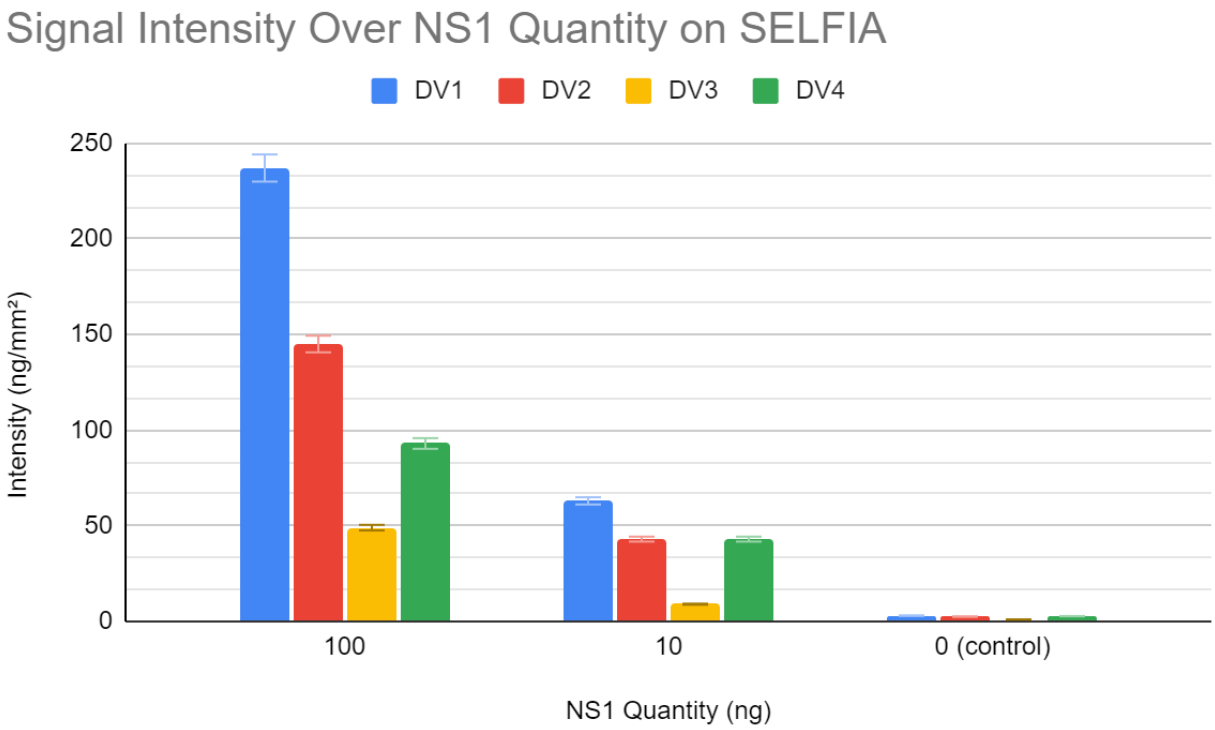


Figure 14. Results of 75ng/μL Rb pAb-FND on lateral flow immunoassay of 100ng and 10ng DV1~4 NS1. The graph shows the comparison of the signal intensity detected on the peaks between LFIA test strips with 100ng, 10ng, and 0ng of all serotypes of NS1 antigen.

From Figure 13, distinct peaks in FND mass are observed in all groups. The signals magnitude's dose-dependency is also shown by the differences in the masses of FNDs produced by the 100ng group and the 10ng group of DV1 to DV4. As seen in Figure 14, with higher concentrations of NS1 immobilized, there is an increase in the signal of (approximately 3.68 times) reported by SELFIA on the Rb pAb-FND-NS1 complex, which indicates a higher association of Rb pAb-FND with the NS1 antigens on the membrane.

Re-optimization

After the initial experimentation, re-optimization was done. Following previous success, we extended our experiment to test the range of detection and evaluated the differences in intensity of signals by altering the quantity of NS1 antigen on the test strip. To do that, we added 100ng, 10ng, 1ng, and 0.1ng of each type of NS1 onto the nitrocellulose membrane and ran Rb pAb-FND solution through the strip to observe the results.

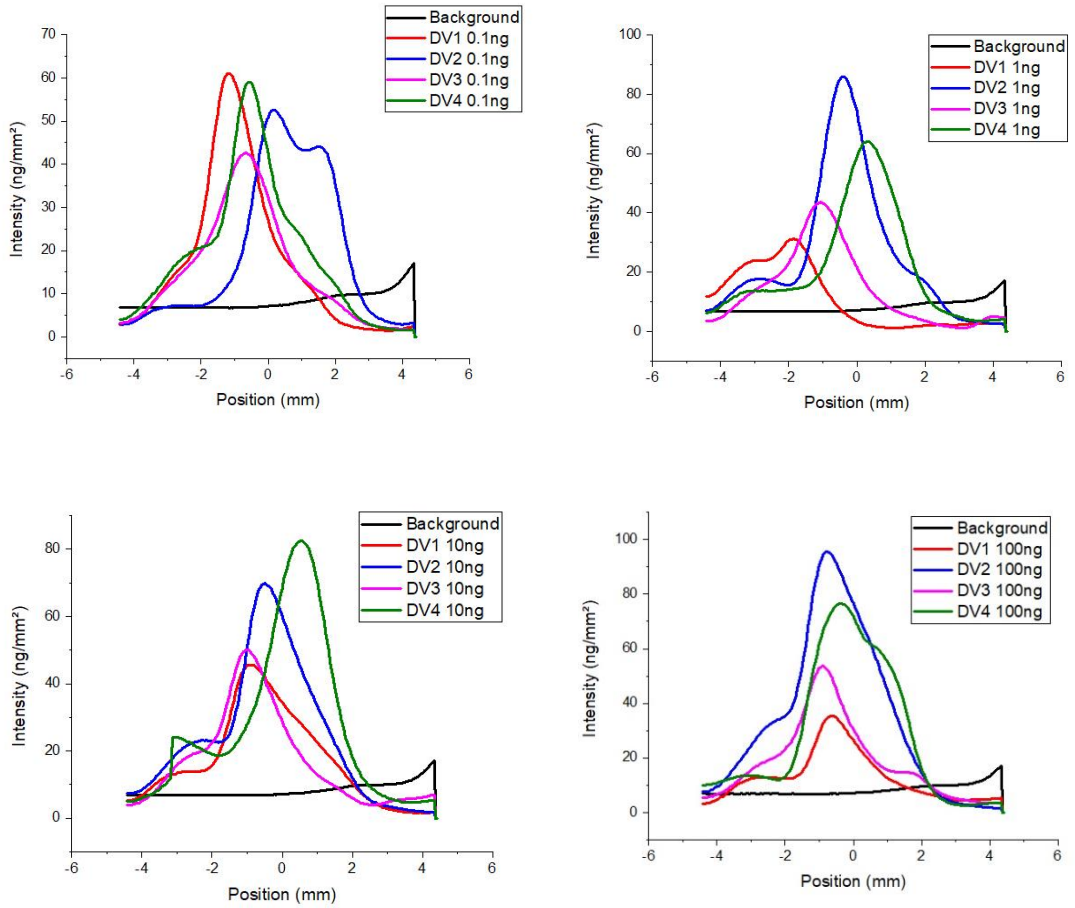


Figure 15. Results of 75ng/µL Rb pAb-FND on lateral flow immunoassay of 100ng, 10ng, 1ng, 0.1ng of all Dengue serotype NS1. The graph shows the comparison of signal intensity detected on its relative positions between LFIA test strips with 100ng, 10ng, 1ng, 0.1ng of all serotype NS1 antigen.

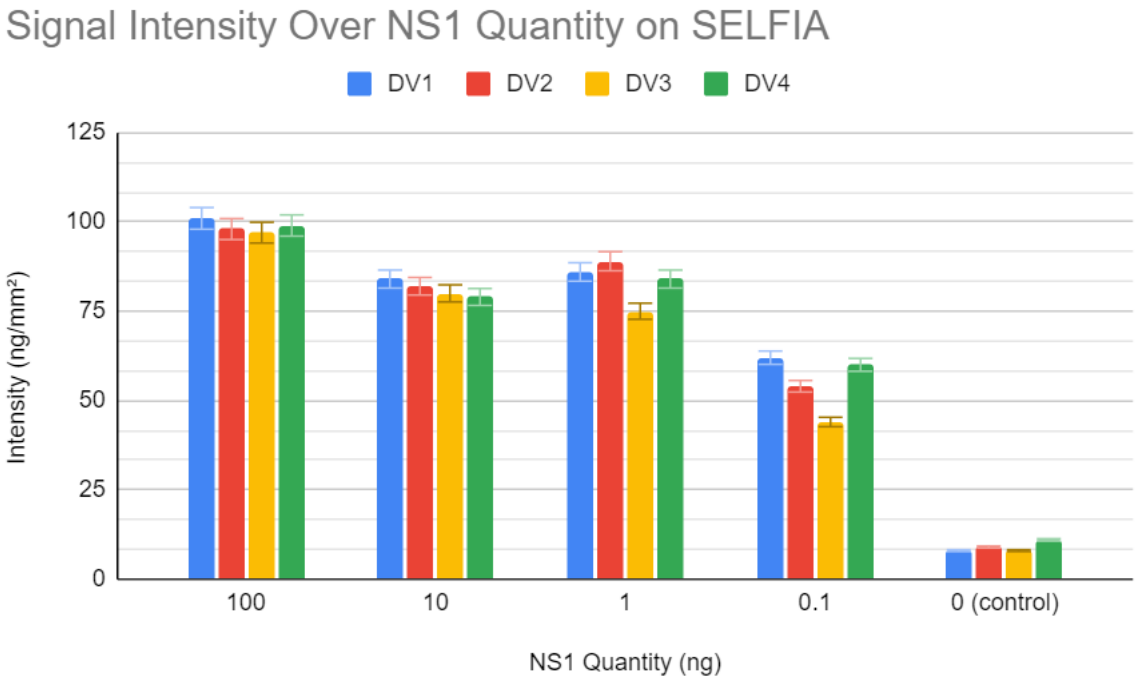


Figure 16. Results of 75ng/μL Rb pAb-FND on lateral flow immunoassay of 100ng, 10ng, 1ng, and 0.1ng DV1~4 NS1. The graph shows the comparison of the signal intensity detected on the peaks between LFIA test strips with 100ng, 10ng, 1ng, 0.1ng, and 0ng of all serotypes of NS1 antigen.

From Figure 15, distinct peaks were not only found in the previous 100ng and 10ng groups, but also the new 1ng and 0.1ng groups. In Figure 16, signals separated from background noises can be observed in all NS1 quantity groups. This indicates SELFIA' s ability to detect Dengue NS1 with a concentration as low as 0.1ng, showing an increased range of detection compared to previous LFIA models.

二、Discussion

LFIA traditionally uses colloidal gold as the indicators of conjugation. Its low price and relatively simple use allow it to be produced commercially. However, colloidal gold has historically been susceptible to more errors, in cases such as pregnancy tests and other application [13-15]. The current methods have limitations in total volume which decreases sensitivity, but with FNDs and SELFIA, insensitivity is reduced.

	Lateral Flow Immunoassay	Spin-Enhanced Lateral Flow Immunoassay
Diagnostic Marker	Nanogold	Fluorescent Nanodiamond
Observation Method	Visual indicator	Magnetically modulated fluorescence
Detection Time	~20 minutes	~20 minutes
Sensitivity	75%	100%
Specificity	98%	100%
Limit of Detection	1ng/μL	0.1ng/μL

Figure 17. Comparison between Lateral Flow Immunoassay and Spin-Enhanced Lateral Flow Immunoassay. The chart lists out several key differences in the mechanism and quality of lateral flow immunoassay and spin enchanted lateral flow immunoassay.

Although FND has higher sensitivity and more accurate conjugation, nanogold has the convenience of naked eye visible results [10]. We were also unable to obtain the equipment to draw multiple lines of antigens on the lateral flow membrane for simultaneous and four-in-one detection of different serotypes of Dengue fever seen in Figure 18. In future studies, different models of lateral flow immunoassays seen in Figure 19 such as sandwich assay, which pairs different antibodies for larger substrates, and competitive assay, which is used for the determination of smaller analytes, can be tested to uncover FND SELFIA's full commercial potential [11].

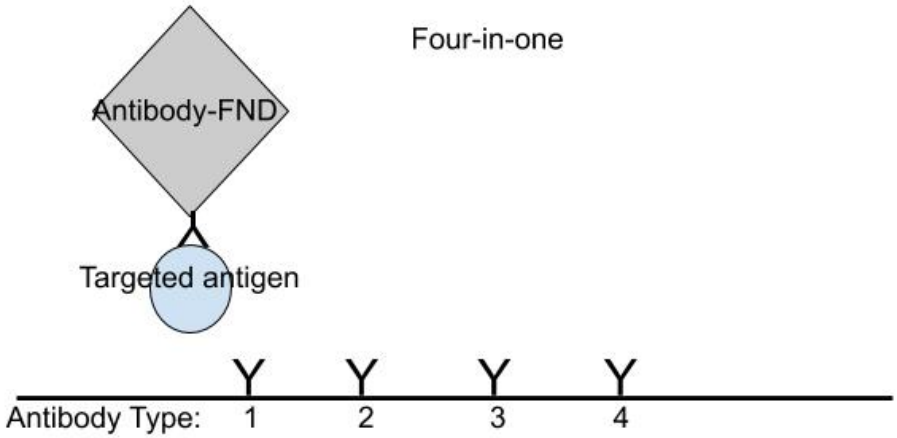


Figure 18. Demonstration of the Four-In-One Assay Models. The diagram shows the proposed model of the four-in-one Dengue SELFIA.

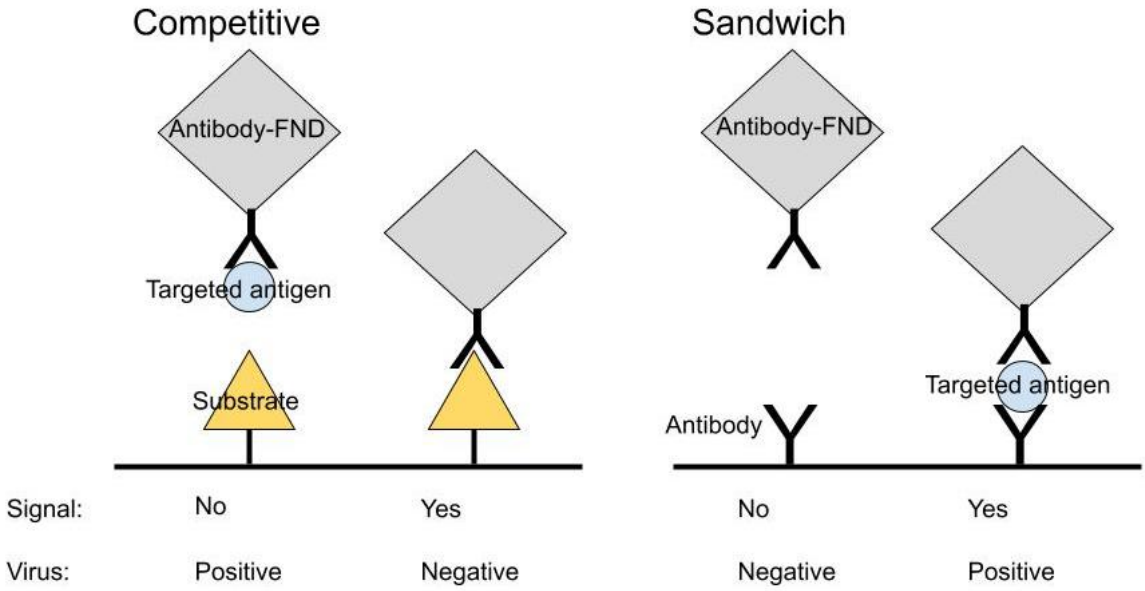


Figure 19. Demonstration of the Competitive Assay and Sandwich Assay Models. The diagram shows the variations between different LFIA methods and their results.

肆、結論與應用

In this investigation, we experimented with fluorescent nanodiamond (FND) as a new diagnostic marker for lateral flow immunoassay (LFIA) of Dengue fever, proposing the new model spin enhanced lateral flow immunoassay (SELFIA) with magnetic modulation [6]. With FND’ s nitrogen-vacancy center, which emits fluorescence when excited by laser [7], we utilized its perks to quantify the signal of detection by installing magnetic modulation and observed its heightened effectiveness in Dengue NS1 antigen detection [6].

We discovered that SELFIA using FND not only serves as a diagnostic marker for LFIA of Dengue fever, but also displays an increased sensitivity of approximately 100 times compared with conventional nanogold-based LFIA [16]. In addition, we also proved SELFIA’ s increased range of detection the enhanced signal results from the magnetic modulation of fluorescence emitted by FNDs during the detection process.

From this study, we have proved FND-based LFIA for Dengue fever to be a novel and ideal diagnostic method because of its high sensitivity and range of detection. Our new findings may provide an improved LFIA for viruses beyond Dengue virus such as the Zika virus or recent COVID-19 and help with the early treatment of diseases by renewing diagnostic methods.

伍、參考文獻

[1]Bhatt, S., Gething, P. W., Brady, O. J. et al. The Global Distribution and Burden of Dengue. Nature 496(7446) (2013) 504-507.

[2]World Health, O., Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control. Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control. Geneva: World Health Organization (2009) 1-147.

[3]Wang, S. F., Wang, W. H., Chang, K. et al.Severe Dengue Fever Outbreak in Taiwan.Am J Trop Med Hyg 94(2016) 193-197.

[4]Guzman, M., Gubler, D., Izquierdo, A.et al.Dengue Infection.Nat Rev Dis Primers2 (2016)16055.

[5]Linares, E. M.,Kubota, L. T.,Michaelis, J.Thalhammer, S.Enhancement of the Detection Limit for Lateral Flow Immunoassays: Evaluation and Comparison of Bioconjugates.J. Immunol. Methods375 (2012)264-270.

[6]Hui, Y. Y., Chen, O. Y., Lin, H. H. et al. Spin-Enhanced Lateral Flow Immunoassay:A Rapid, Quantitative, and Ultrasensitive DiagnosticPlatform. NChem (2020) 1-21.

[7]Hsiao, W. W. W., Hui, Y. Y., Tsai, P. C. et al. Fluorescent Nanodiamond: A Versatile Tool for Long-term Cell Tracking, Super-resolution Imaging, and Nanoscale Temperature Sensing. Acc Chem Res 49 (2016)400-407.

[8]Libraty, D. H.,Young, P. R.,Pickering, D. et al.High Circulating Levels of the Dengue Virus Nonstructural Protein NS1 Early in Dengue Illness Correlate with the Development of Dengue Hemorrhagic Fever.J. Infect. Dis.186 (2002)1165-1168.

[9]Morens, D. M.Dengue Fever and Dengue Hemorrhagic Fever.Pediatr. Infect. Dis. J.28 (2009)635-6.

[10]Screaton, G., Mongkolsapaya, J., Yacoub, S.et al.New Insights Into the Immunopathology and Control of Dengue Virus Infection.Nat Rev Immunol15 (2015) 745-759.

[11]Cheng, C. Small-volume Point-of-care Analytical Methods.Sci Rep10 (2020)14230.

[12]Koczula, K. M., Gallotta, A.Lateral flow assays.Essays in Biochemistry(2016)60(1):111-120.

[13]Jiang, X., Lillehoj, P.B. Microneedle-based Skin Patch for Blood-free Rapid Diagnostic Testing.Microsyst Nanoeng6 (2020)96.

[14]Quesada-González, D., Jairo, G.A., Blake, R.C.et al.Uranium (VI) Detection in Groundwater Using a Gold Nanoparticle/Paper-based Lateral Flow Device.Sci Rep8 (2018)16157.

[15]Srisrattakarn, A., Tippayawat, P., Chanawong, A. et al. Development of a Prototype Lateral Flow Immunoassay for Rapid Detection of Staphylococcal Protein A in Positive Blood Culture Samples.Diagnostics10 (2020) 794.

[16]Zhang, Y., Liu, X., Wang, L.et al.Improvement in Detection Limit for Lateral Flow Assay of Biomacromolecules by Test-Zone Pre-enrichment.Sci Rep10, (2020)9604.

[17]Tsai, T., Huang, T., Chen, C.et al.Development a Stacking Pad Design for Enhancing the Sensitivity of Lateral Flow Immunoassay.Sci Rep8 (2018)17319.

[18]Su, L.J., Wu, M. S., Hui, Y. Y. et al. Fluorescent Nanodiamonds Enable Quantitative Tracking of Human Mesenchymal Stem Cells in Miniature Pigs. Sci. Rep. 7 (2017) 45607.

[19]Chang, B. M., Lin, H. H., Su, L. J. et al. Highly Fluorescent Nanodiamonds Protein-functionalized for Cell Labeling and Targeting. Adv Funct Mater 23 (2013) 5737-5745.

[20]Fu, C. C., Lee, H. Y., Chen, K. et al. Characterization and Application of Single Fluorescent Nanodiamonds as Cellular Biomarkers. Proc Natl Acad Sci USA 104 (2007) 727-732.

[21]Igarashi R., Yoshinari Y., Yokota H. et al. Real-time Background-free Selective Imaging of Fluorescent Nanodiamonds in Vivo. Nano Lett 12 (2012) 5726-5732.

[22]Kuo Y., Hsu T. Y., Wu Y. C., Chang H. C. Fluorescent Nanodiamond as a Probe for the Intercellular Transport of Proteins in Vivo. Biomaterials34 (2013) 8352.

[23]Hsu, T., Liu, K., Chang, H.et al.Labeling of Neuronal Differentiation and Neuron Cells with Biocompatible Fluorescent Nanodiamonds.Sci Rep4 (2014)5004.

[24]Cordina, N.M., Sayyadi, N., Parker, L.M.et al.Reduced Background Autofluorescence for Cell Imaging Using Nanodiamonds and Lanthanide Chelates.Sci Rep8 (2018)4521.

[25]Hui, Y., Su, L., Chen, O.et al.Wide-field Imaging and Flow Cytometric Analysis of Cancer Cells in Blood by Fluorescent Nanodiamond Labeling and Time Gating.Sci Rep4 (2014)5574.

【評語】090015

作者先是優化 pAb-FND 於 4 stereotypes of NS1 的檢測力。接著探討其對不同濃度 DV1-4 的敏感性，並聲稱此法具有準確性。確實抗原量對於免疫檢測是為一大限制，作者在此提供一個新的檢測方式大幅增加其靈敏性。以臨床來說的確可能可以增加確診率已達到預防之成效，希望作者能多加討論此檢測方法是否對特定血清型有所偏好。另外此法是否能推廣尚是個問題，畢竟相比傳統 LFIA 還需要額外器材才能判斷 POS or NEG。此研究雖尚有普及及成本疑慮，但總體而言已有雛型，值得期待。此作品有應用淺力，但準確度和重現性需再優化。